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19. (Amended) The method as in Claim 18 wherein the subject is a human suffering from gastroparesis, gastroesophageal reflux disease, anorexia, gall bladder stasis, postoperative paralytic ileus, scleroderma, intestinal pseudoobstruction, gastritis, emesis, [and] or chronic constipation (colonic inertia).

REMARKS

The Invention

The invention provides motilide compounds useful in the treatment of conditions characterized by impairment of gastric motility. The inventive compounds are semi-synthetic derivatives of erythromycin wherein the erythromycin 13-ethyl group is replaced with *n*-propyl. Surprisingly, the compounds provided by the present invention effective at stimulating gastric motility with without measurable induction of antibiotic activity. Thus, the compounds of the invention are useful for the treatment of such digestive disorders as gastroparesis and gastro-esophageal reflux disease.

Response to Restriction Requirement

The Applicants confirm the previous election of Group I. The Applicants understand that the restriction requirement shall be withdrawn upon the allowance of Claims 18 and 19.

The Amendments to the Specification

The Specification is amended to correct typographical errors that would be apparent to one of skill in the art and update the status of a referenced pending U.S. patent application. Therefore, the Applicants believe that the amendments to the Specification do not add any new matter to this application.

On page 12, line 13, the wording of the Markush group for R^8 has been corrected to add the missing "or" between the last two members of the group.

On page 18, line 17, the Attorney Docket number has been replaced by the serial number for the cited patent application.

On page 20, line 14, the misspelling "erythromcyins" has been corrected.

On page 27, line 20, the typographical error "Two embodiment" has been corrected.

The Amendments to the Claims

Claims 3–6 were cancelled without prejudice to further prosecution in a related application, including without limitation: a divisional, continuation, or continuation-in-part application.

Claims 1 was amended to recite compounds of the invention that include saturated carbon atoms as the 6–9 positions and for which R^7 is *n*-propyl. Claim 2 was amended to conform with claim 1. Claim 7 was amended to depend from, and conform to, Claim 1. Support for these amendments can be found, for example, at pages 55–57. Claims 8–11 were amended to recite specific embodiments of the invention in graphical form. Claim 18 was amended to depend from Claim 1 and to recite the step of administering a therapeutically effective amount of a composition comprising a compound of the invention. Support for these amendments can be found in the corresponding claims as filed originally. Claim 19 was amended to correct a minor error. Therefore, the Applicants believe that the amendments to the claims do not add any new matter to this application.

Rejections

In the Office Action mailed July 10, 2002, Examiner Elli Peselev rejected pending Claims 1–15 and 18–19. The bases for the rejections and the Applicants' responses thereto are set forth below.

The Rejections Under 35 U.S.C. § 112

Claims 18 and 19 were rejected under 35 U.S.C. § 112, allegedly for failing to particularly point out and distinctly claim the subject matter which applicant regards as the invention. More specifically, the Examiner rejected Claim 18 for allegedly being unclear in describing whether the composition of the invention is administered to a subject in need of treatment. The Examiner rejected Claim 19 for allegedly being unclear for including improper Markush terminology. The Applicants respectfully submit these rejections are moot in view of the pending claims, and, therefore, respectfully request the Examiner to withdraw the rejections.

The Rejections Under 35 U.S.C. § 103(a)

The Examiner rejected Claims 1–15 and 18–19 under 35 U.S.C. § 103(a), allegedly for being unpatentable over Freiberg *et al.* (U.S. Patent 5,538,961) in view of Lundy *et al.* (U.S. Patent 6,043,226). The Applicants traverse these rejections respectfully in view of the following remarks.

The pharmacology and medicinal chemistry arts recognize that the defining feature of motilde activity is the ability of a compound to possess prokinetic activity *but not antibacterial activity*. *See*, Peeters, T.L., "Erythromycin and Other Macrolides as Prokinetic Agents", *Gasteroenterology* 105:1886–1899 (1993), at 1887; Faghih, R., *et al.*, "Motilides and motilactides: design and development of motilin receptor agonists as a new class of gastrointestinal prokinetic drugs", *Drugs of the Future*, 23(8): 861–872 (1998), at 862; and Clark, *et al.*, "Erythromycin Derivatives ABT229 and GM611 Act On Motilin Receptors in the Rabbit Duodenum", *Clin. Expr. Pharm. Phys.* 26:242–245 (1999) at 242. (Copies submitted herewith.)

The compounds of the present invention possess the requisite properties of motilides: they possess prokinetic activity *without* antibacterial activity. The attached Declaration from Robert G. Johnson, M.D., Ph.D., demonstrates that representative compounds of the invention possess the ability to induce contractions in rabbit duodenum muscle strips that is comparable with the action of motilin. However, the representative compounds tested do not have antibacterial activity. Thus, the compounds of the invention indeed meet the requisite properties of motilides. Moreover, the representative compounds of the invention demonstrated oral bioavailability, an important property in compounds used as prokinetic agents.

In contrast, the Applicants respectfully submit that the combination of Freiberg and Lundy suggested by the Examiner fails to support a rejection for obviousness. According to M.P.E.P. § 2143, a *prima facie* case of obviousness requires three basic criteria:

(1) there must be some suggestion or motivation to modify or combine the references, either in the references themselves or in the general art;
(2) there must be a reasonable expectation of success; and
(3) the prior art reference(s) must teach or suggest all the claim limitations.

The fact that references can be combined or modified does not render the resultant combination obvious unless the prior art also suggests the desirability of the combination.

The Freiberg reference discloses macrocyclic lactams derived from erythromycin, along with intermediates thereto and methods for their preparation. As recognized by the Examiner, Freiberg teaches compounds having an ethyl group at the 13-position. The Freiberg reference teaches that the disclosed compounds are devoid of antibacterial activity (see column 4, line 26). The Freiberg reference does not show or suggest alteration at the 13-position of the central ring.

The Lundy reference discloses antibacterial compounds that are C9-modified derivatives of erythromycin that optionally comprise a cyclic 3, 6-acetal moiety. Furthermore, the

compounds disclosed in Lundy *et al.* are antibacterial or anticancer compounds. Lundy, *et al.*, do not show or suggest compounds having motilide activity. The Lundy reference does not show or suggest any specific alteration at the 13-position of the central ring, but, rather, asserts a large range of substituents at that position, including "α-branched C_3–C_6 alkyl" at column 4, line 56.

Thus, neither Lundy, *et al.*, or Freiburg, alone or in combination, show or suggest the *n*-propyl erythromycin derivatives of the invention, and, therefore, do not show or suggest all of the claim elements.

Furthermore, the combination of the teachings of Freiberg and Lundy would not provide a reasonable expectation to one having skill in the art that the results of the claimed motilide compounds can be achieved. Indeed, the Applicants respectfully submit that those having skill in the medicinal chemistry arts understand that the effect of any structural modification of an erythromycin derivative on motilide activity is unpredictable. According to Sarna *et al.*, "Enteric locus of action of prokinetics: ABT-229, motilin, and erythromycin," *Am. J. Physiol. Gastrointest. Liver Physiol.* (2000) *278*: G744-G752:

> One class of prokinetic agents that has received attention during the last few years is motilides (28). These molecules, including ABT-229, are modifications of the macrolide molecule but are devoid of antibiotic activity. Whereas the molecular modification retains the ability to of the new molecule to stimulate contractions, the efficacy and potency may be different from that of the original molecule. *More importantly, it is not known whether even subtle molecular modification alters the site of action and receptors involved in stimulating gut contractions.* Such alteration may account for differences in potency and efficacy and organ specificity of motilides. (Emphasis added.)

There the Applicants respectfully submit that no basis exists in the medicinal chemistry and pharmacology arts for an expectation of successfully making the motilide compounds of the invention by combining the vaguely-defined variations at the 13-position as described for antibacterial agents in the Lundy reference with the motilide macrolactam

structures described in Freiberg *et al.*, either in the cited references or in the general art at the time the instant invention was made.

Moreover, those having skill in the medicinal chemistry arts understand that compounds used as motilides should be substantially devoid of any antibacterial activity. The medical and medicinal chemistry arts recognize generally that antibiotics should be reserved for treating bacterial infections to reduce the induction of bacterial resistance to antibiotics. Guerin, *et al.*, "Why Not to Use Erythromycin in GI Motility", *Chest* 121(1): 301 (January, 2002), at 301. (Copy submitted herewith).

The Applicants respectfully submit that Lundy's teachings are directed solely to compounds having antibacterial activity (see Lundy at columns 26–30); thus, one having ordinary skill in the medicinal chemistry arts would expect compounds incorporating features described by Lundy would have antibacterial activity. Lundy, therefore, teaches away from the invention and from the requirements of motility agents as known in the medicinal chemistry and pharmacology arts.

Thus, one having skill in the medicinal chemistry arts would neither be motivated to combine Lundy with Freiberg nor expect the results of the present invention from such a combination. The Applicants therefore respectfully request the withdrawal of all rejections under 35 U.S.C. § 103(a).

Summary

Amendments to the claims have been submitted in response to the Office Action mailed July 10, 2002. The Applicants hereby request favorable reconsideration of the application in view of the foregoing amendments and remarks and prompt allowance of all claims and passage of the application to issue.

In the unlikely event that the transmittal letter is separated from this document and the Patent Office determines that an extension and/or other relief is required, the applicants hereby petition for any required relief, including extensions of time, and authorize the Assistant Commisioner to charge the cost of such petitions and/or other fees due in connection with the filing of this document to **Deposit Account No.** _____ referencing docket no. 010041.02.

Respectfully submitted
KOSAN BIOSCIENCES, INC.

David P. Lentini
Registration No. 33,944

Date: January 3, 2003

PATENT
Docket No. 010041.02

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In the application of:

Gary ASHLEY, *et al.*

Serial No.: 09/990,554

Filing Date: November 21, 2001

For: MOTILIDE COMPOUNDS

Examiner: Elli Peselev

Group Art Unit: 1623

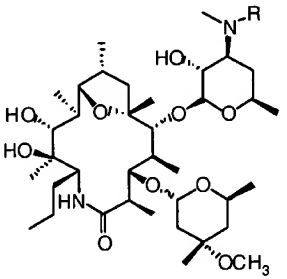
Declaration of
Robert G. Johnson, M.D., Ph.D.
Pursuant to 37 C.F.R. § 1.132

I, Robert G. Johnson, M.D., Ph.D., state and declare as follows:

1. I am Senior Vice President, Medical Affairs and Corporate Development and head of the pharmacology group of Kosan Biosciences, Inc. I received my M.D. and Ph.D. from the University of Pennsylvania. Subsequently, I was on the faculty at Harvard Medical School (1985–1987) and at the University of Pennsylvania (1987–1991). Prior to joining Kosan, I was Director of Pharmacology at Merck & Co. (1991–1998), Vice President for Pharmacology and Preclinical Affairs (1998–2000), and Vice President for Corporate Development (2000) at Chiron Corporation.

2. I am not an inventor in the above-identified patent application.

3. Dr. Daniel Santi, told me that he expected the compounds shown below to have motilide activity. Dr. Santi asked me to test the these compounds for motilide activity:



R = Me compound A
R = Et compound B
R = ^{i}Pr compound C

4. I instructed scientists under my supervision to perform tests relating to motilin receptor activation, antibacterial activity, drug bioavailability, and drug half-life on one or more of the above compounds. These tests are known in the art, and are accepted as pre-clinical indicators of utility as motilides. The results of these tests are provided in Exhibit A below.

5. Compounds A, B, and C showed potent activation of cloned human motilin receptor expressed in cultured cells, as measured using a calcium-influx assay described in PCT publication WO02/04462 (see Table 1). This assay provides an estimate of the potency of a compound as a motilide. Compounds A, B, and C were significantly more potent motilides than erythromycin A as measured by this *in vitro* test.

6. MIC values were determined by MDS Pharma Services, Bothell, WA, by inoculating nutrient broth containing from 0.03 μg/mL to 100 μg/mL of test compound with ATCC6301, allowing the cultures to grow for 1 to 4 days, and measuring cell growth by the turbidity of the culture. The MIC is the minimal concentration at which cell growth is prevented. While erythromycin A prevented growth of the bacterium at a concentration of 0.03 μg/mL, Compounds A, B, and C showed no inhibition of bacterial growth at the maximum concentration tested, giving an MIC greater than100 μg/mL. Compounds A, B, and C thus showed no antibacterial activity as demonstrated by measurement of the minimal inhibitory concentration (MIC) against cultured *Streptococcus pneumoniae* ATCC6301, a strain which is sensitive to erythromycin A.

7. Rat pharmacokinetics were measured by Charles River Laboratories, Worcester, MA, and compared plasma levels of the compound when administered either by intravenous injection or per oral gavage. Compounds were formulated in ethanol/5% lactobionate/saline (1:1:48 v/v/v) at a final concentration of 1 mg/mL. Male Sprague-Dawley rats equipped with surgically-implanted jugular vein catheters to facilitate i.v. dosing were placed into groups of three animals per group. Each group was given one compound either by i.v. administration or by oral gavage at a target dose of 2 mg/kg.

Blood samples were collected prior to dosing and periodically up to 24 h after dosing. Blood samples were centrifuged to obtain plasma, which was flash frozen in liquid nitrogen. Compound concentrations were determined by LC/MS-MS. A comparison of the peak plasma concentrations between the i.v. and p.o. administrations showed that Compound C has a bioavailability of 24% in the rat and a half-life of 1.9 hours. Compound C showed good bioavailability in both rat and dog, as measured by peak plasma concentrations. In contrast, erythromycin A had a bioavailability of 13% and a half-life of 1 hour in the rat.

8. Dog pharmacokinetics were measured by Charles River Laboratories, Worcester, MA, and compared plasma levels of the compound when administered either by intravenous injection or per oral gavage to male beagles. Compounds were formulated in ethanol/5% lactobionate/saline (1:1:48 v/v/v) at a final concentration of 1 mg/mL. Male beagles equipped with a temporary percutaneous catheter placed in a saphenous vein to facilitate i.v. dosing were placed into groups of two animals per group. Each group was given one compound either by i.v. administration at a target dose of 0.5 mg/kg or 1.5 mg/kg or by oral gavage at a target dose of 1 mg/kg or 3 mg/kg. Blood samples were collected prior to dosing and periodically up to 24 h after dosing. Blood samples were centrifuged to obtain plasma, which was flash frozen in liquid nitrogen. Compound concentrations were determined by LC/MS-MS. A comparison of the peak plasma concentrations between the i.v. and p.o. administrations showed that Compound C has a bioavailability of 65% in the dog and a half-life of 3.6 hours.

9. These pharmacokinetic data are consistent with the determination that Compound C is suitable for oral administration as a motilide.

10. I also instructed scientists under my supervision to test the compounds for their ability to affect muscle strip contractility. The muscle strip contractility data (shown in Exhibit B) was obtained by Dr. Theo L. Peeters, Director of the Gut Hormone Laboratory at the Center for Gastroenterological Research, University of Leuven, Belgium, at my request. The experiments were performed as described in Depoortere *et al.*, "The erythromycin

derivative EM523 is a Potent Motilin Agonist in Man and in Rabbit," *Peptides 11*, 515-519 (1990). Panel A of Exhibit B shows the contractility results of a control experiment using 100 nM motilin on rabbit duodenum muscle strips. The graphs show the contraction of the muscle strip in millimeters as a function of time in seconds. Treatment of the tissue with 100 μM acetylcholine (labeled "Ach E-4") at the beginning and end of each experiment was used to calibrate the maximum contractility of the muscle, which varied between preparations as well as during the period of the experiment. Treatment of the tissue with 30 nM motilin induced maximal contraction. Repeated doses of motilin, indicated on the graph by arrows, were provided following washing of the strips to remove the previous dose and showed progressively smaller effects, presumably due to tiring of the muscle as indicated by the decreased response to acetylcholine at the end of the experiment.

11. Panels B, C, and D of Exhibit B show the results of the corresponding muscle contractility tests with Compounds A, B, and C, respectively. Again, each muscle strip was calibrated at the beginning and end of each experiment by measuring the effect of 100 μM acetylcholine. Treatment of the tissue strips with 100 nM concentrations of each of compounds A, B, and C induced contractions, with Compound C appearing to have greater potency . Repeated doses after removal of previous doses by washing of the strips, again indicated by arrows, showed progressively smaller effects, again presumably due to tiring of the muscle. The effects of these compounds on rabbit duodenum muscle tissue in this test are thus comparable to that of the natural motility hormone motilin.

12. Based on the above described test results, I believe that the compounds tested, in particular Compound C, show the preclinical features expected of an agent having clinically useful motilide properties.

13. I hereby declare that all statements made herein of my knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements are made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the

United States Code and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

Robert Johnson, M.D., Ph.D.

December 19, 2002
Date

EXHIBIT A

Test Results for Compounds A, B, and C

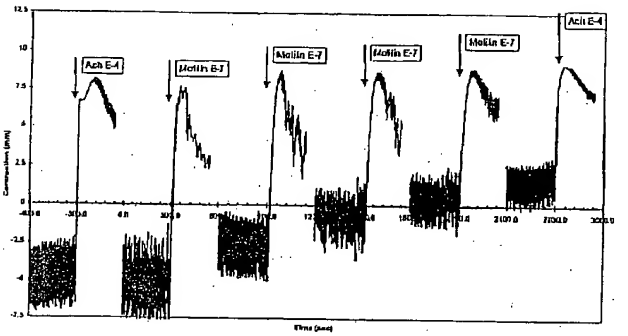
Compound	Receptor Activation EC_{50}(nM)	Antibacterial Activity MIC(μg/mL)	Bioavailability		$T_{1/2}$	
			rat	dog	rat	dog
Erythromycin A	1,300	0.03	13%	n.d.	1h	n.d.
A	97	> 100	n.d.	n.d.	n.d.	n.d.
B	69	> 100	n.d.	n.d.	n.d.	n.d.
C	26	> 100	24%	65%	1.9h	3.6h

n.d. = not determined

EXHIBIT B

Muscle Strip Contractility Assays

A. Motilin Control at 100 nM



B. Compound A at 100 nM

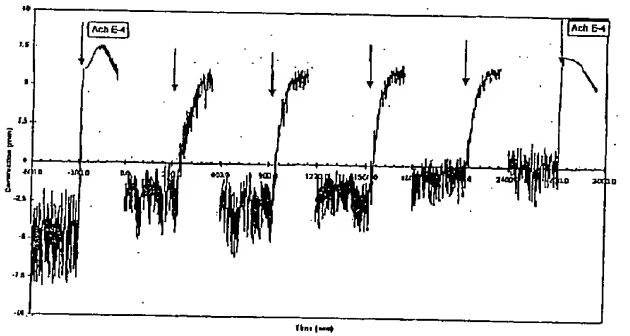
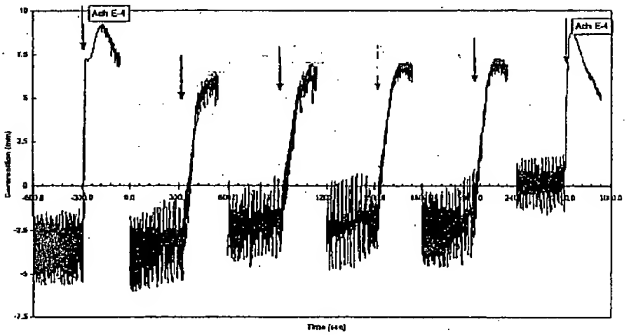
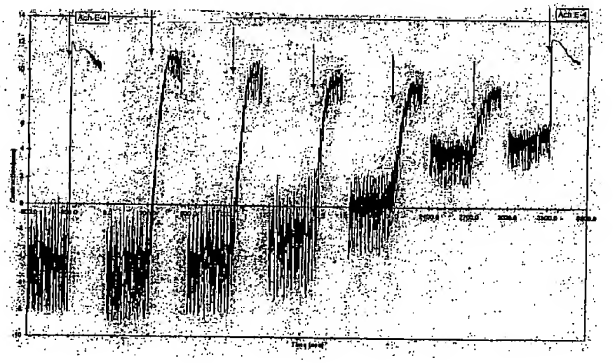


EXHIBIT B (cont.)

C. Compound B at 100 nM



D. Compound C at 100 nM



Clinical and Experimental Pharmacology and Physiology (1999) 26, 242–245

ERYTHROMYCIN DERIVATIVES ABT 229 AND GM 611 ACT ON MOTILIN RECEPTORS IN THE RABBIT DUODENUM

MJ Clark,* T Wright,† PP Bertrand,† JC Bornstein,† KM Jenkinson,* M Verlinden‡ and JB Furness*

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SUMMARY

1. The present study was undertaken to determine whether the macrolide antibiotic erythromycin, its stable motilide derivatives ABT 229 and GM 611 and motilin act at the same receptors on intestinal muscle

2. Each compound contracted the longitudinal muscle of the rabbit duodenum in a concentration-dependent manner that was unaffected by 1 μmol/L tetrodotoxin. The potency order (pEC_{50} values in brackets) was motilin (8.4), ABT 229 (7.6), GM 611 (7.5) and erythromycin (6.0).

3. The motilin receptor antagonists GM 109 and [phe^3,leu^{13}]-motilin, both shifted the concentration–response curves for each agonist to the right, but did not affect concentration–response relationships for the muscarinic agonist carbachol. Schild regression analysis yielded similar pA_2 values for GM 109 (in the range 7.2–7.5) for all agonists. This analysis was not done for [phe^3,leu^{13}]motilin, which was a non-competitive antagonist and partial agonist.

4. It is concluded that erythromycin, the motilides and motilin act at the same (motilin) receptor on rabbit duodenal muscle and do not have any detectable actions at other receptors in this preparation.

Key words: intestine, motilides, motilin, prokinetics.

INTRODUCTION

The duodenal hormone motilin is a stimulant of gastric and small intestinal motility in several species, including humans.[1,2] Injection of motilin initiates phase III of the migrating motor complex in humans and dogs and causes contraction of isolated gastrointestinal muscle from rabbit and human.[2] The rabbit duodenum, which is more effectively contracted by motilin than are other gastrointestinal tissues *in vitro*, has become a standard preparation for characterizing motilin receptors and agents that act at motilin receptors.[3]

In 1984, Itoh *et al.*[4] discovered a motilin-like action of the macrolide antibiotic erythromycin, which elicited strong phasic contractions throughout the gastrointestinal tract when it was injected into dogs. Subsequent *in vitro* binding and contractility studies provided evidence that erythromycin acts on the motilin receptor.[1] This discovery was followed by a search for orally acting erythromycin analogues that lack antibiotic activity but retain agonist potency at the motilin receptor and could, therefore, be used as prokinetic agents. These erythromycin derivatives are termed 'motilides'.

Low bioavailability was a problem with several of the early compounds, such as EM523, EM574 and EM536, which were rapidly degraded in the acidic conditions in the stomach.[5,6] More recently, stable motilides, including ABT 229 and GM 611, have been developed by protection from spirochaetal formation (which results in the degradation of these molecules) via the removal (ABT 229) or methylation (GM 611) of the carbon-12 hydroxylation site.[5,6]

ABT 229 is a potent agonist that accelerates gastric emptying in humans.[7,8] However, not all gastrointestinal sites at which erythromycin acts are motilin receptors[9] and, thus, erythromycin derivatives cannot automatically be presumed to act at motilin receptors. Thus, despite evidence that ABT 229 displaces bound motilin in binding studies,[3] adequate pharmacological evidence for it acting on the same receptors as motilin on gastrointestinal muscle is not available. The limiting factor in pharmacological studies to date has been the lack of availability of suitable motilin receptor antagonists. The present study uses two antagonists, [phe^3,leu^{13}]motilin, a modified version of the full motilin molecule, and GM 109, a cyclic tetrapeptide derived from the first four amino acid residues of motilin. Both have been reported to lack agonist properties in rabbit isolated duodenum, although [phe^3,leu^{13}]motilin is a full agonist in chicken isolated intestine.[9,10]

The aim of the present study was to determine whether these motilin receptor antagonists affect motilin, erythromycin and ABT 229 similarly in the rabbit duodenum and, thus, whether ABT 229 and GM 611 act solely at motilin receptors in this tissue.

METHODS

Tissue preparation

New Zealand white rabbits of either sex (weighing 2–3 kg) were anaesthetized with 40 mg/kg pentobarbitone (Nembutal; Boehringer Ingelheim, Artarmon, NSW, Australia) and were killed by severing the spinal cord and exsanguination. A segment of the duodenum was rapidly removed through a midline incision, washed and freed of mesenteric attachment. The segment was opened along the mesenteric border and gently stripped of the mucosa and submucosa. Muscle strips, approximately 2 cm in length, were mounted longitudinally in organ baths containing 6 mL Krebs' solution (pH 7.4) of the following composition (in mmol/L): NaCl 118.0; KCl 4.8; NaH_2PO_4 1.0; $NaHCO_3$ 25.0; $MgSO_4$ 1.2; D-glucose 11.0; $CaCl_2$ 2.5. The solution was maintained at 36°C and bubbled continuously with a mixture of 95% O_2 and 5% CO_2.

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Received 26 August 1998; revision 19 October 1998; accepted 26 October 1998.

Measurement of contraction

Muscle strips were placed under a resting tension of 1 g. Contractile activity was recorded using Harvard isotonic transducers (South Natick, MA, USA) and the output was monitored by a computerized polygraph system (Biopac Systems, Santa Barbara, CA, USA).

Experimental protocol

Following a 90 min equilibration period, during which time the bath solution was changed every 15–20 min, tissues were exposed three times to 10 μmol/L carbachol, which produced equal responses to each, or to the last two, applications. Cumulative concentration–response curves for human/porcine motilin (referred to as motilin from here on; 0.1 nmol/L to 1 μmol/L), erythromycin (100 nmol/L to 30 μmol/L), ABT 229 (0.1 nmol/L to 1 μmol/L), GM 611 (1 nmol/L to 3 μmol/L) and carbachol (1 nmol/L to 30 μmol/L) were established by adding increasing concentrations of each compound.

The effects of antagonists on agonist concentration–response curves were determined by incubating muscle strips with different concentrations of GM 109 (0.1–3 μmol/L) or [phe^3,leu^{13}]motilin (10–300 nmol/L) for 10 min prior to obtaining an agonist concentration–response curve. Following a 60 min tissue recovery period, during which time the bath solution was changed every 15–20 min, a final response to 10 μmol/L carbachol was obtained. This was almost always the same as the response obtained before the agonist was tested. In order to test whether these compounds act at the muscle or on neurons, concentration–response curves to each of motilin, erythromycin, ABT 229 and GM 611 were obtained in the presence of 1 μmol/L tetrodotoxin (TTX).

Data analysis

Results are expressed as percentages of the amplitudes of contractions evoked by 10 μmol/L carbachol (responses before and after application of the agonist under test were averaged). The pEC_{50} (negative logarithm of the agonist concentration that caused half-maximal contraction) was determined and the pA_2 value for GM 109 as an antagonist against each agonist was determined according to the method of Arunlakshana and Schild.[12] All data are expressed as the mean±SEM.

Materials

The following drugs were used: ABT 229 lactobionate (Abbott Laboratories, Chicago, IL, USA), carbachol chloride (Merck Pharmaceuticals, Philadelphia, PA, USA), erythromycin lactobionate (Abbott), GM 611 (Chugai Pharmaceuticals, Shizuoka, Japan), GM 109 (Chugai), [phe^3,leu^{13}]motilin (Auspep, Melbourne, Victoria, Australia), human/porcine motilin (Auspep) and TTX (Alomone Labs, Jerusalem, Israel).

RESULTS

Motilin, ABT 229, GM 611, erythromycin and carbachol each elicited concentration-dependent contractions of the rabbit duodenal muscle with pEC_{50} values of 8.4±0.1, 7.6±0.1, 7.5±0.1, 6.0±0.1 and 6.8±0.2, respectively. All motilin-like agonists gave similar maxima, approximately 80% of the maximal response to carbachol. The maxima were 77.1±3.3 (motilin), 80.1±6.9 (ABT 229), 76.8±3.1 (GM 611) and 78.8±3.7 (erythromycin). Contractions to motilin, erythromycin, ABT 229 and GM 611 were achieved within 30 s of addition and were sustained in the continued presence of the agonist. The contractions did not subside to baseline on washout of agonist, even after 60–90 min. Therefore, experiments were performed on paired muscle strips, with a single curve constructed in each tissue strip, one with antagonist present and one without. The concentration–response curves for each agonist were unaffected by the presence of 1 μmol/L TTX (Fig. 1).

[Phe^3,leu^{13}]motilin acted as a non-competitive antagonist in these experiments. It also had partial agonist activity; at 300 nmol/L it had maximum agonist effect and caused 10–30% of the contraction evoked by motilin. [Phe^3,leu^{13}]motilin shifted the concentration–response curves to motilin, erythromycin, ABT 229 and GM 611 to the right in a concentration-dependent manner, which was accompanied by a depression in maximum response (Fig. 2a–d) but, up to a concentration of 300 nmol/L, [phe^3,leu^{13}]motilin had no effect on the concentration–response curve to the muscarinic agonist carbachol (Fig. 2e).

GM 109 had no contractile effect at any concentration but, at high concentrations (1 and 3 μmol/L), it caused a slight decrease in muscle tone of some tissue strips. This antagonist shifted concentration–response curves to motilin, erythromycin, ABT 229 and GM 611 to the right in a concentration-dependent manner, with no accompanying change in maximum response (Fig. 3a–d). Up

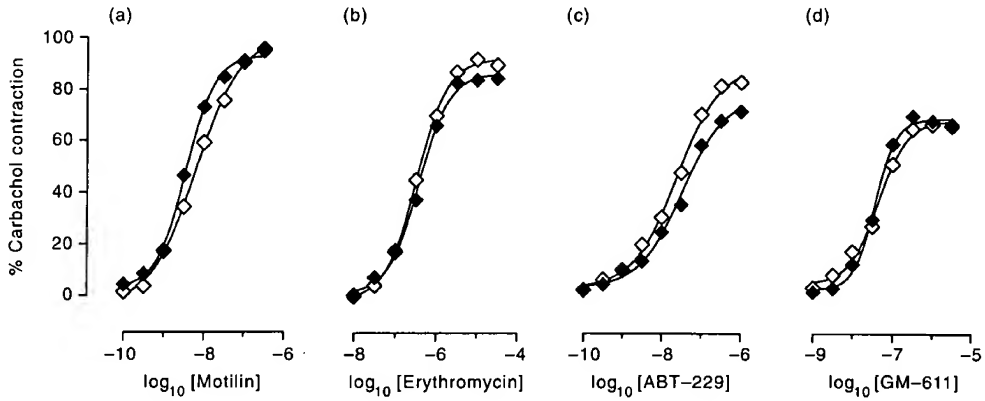

Fig. 1 Concentration–response relationships for contractions of the longitudinal muscle of the rabbit duodenum by motilin (a), erythromycin (b), ABT-229 (c) and GM-611 (d) in the absence (filled symbols) and presence (open symbols) of 1 μmol/L tetrodotoxin (each $n = 1$).

to a concentration of 3 μmol/L, GM 109 had no effect on the concentration–response curve to carbachol (Fig. 3e).

Schild regression analysis for GM 109 as an antagonist yielded similar pA_2 values for each agonist: 7.19 (motilin; slope = 0.65±0.18), 7.54 (erythromycin; slope = 0.67±0.15), 7.32 (ABT 229; slope = 0.73±0.21) and 7.29 (GM 611; slope = 0.91±0.14).

DISCUSSION

The only gastrointestinal tissues where motilin is known to be effective *in vitro* are muscle strips from human and rabbit.[13,14] Contraction of the rabbit duodenum by motilin is not affected by blocking action potentials in neurons with TTX or by blocking the actions of excitatory transmitters with hexamethonium or atropine.[14,15] Moreover, motilin does not modify transmission from excitatory neurons in the rabbit ileum.[14] Therefore, it has been concluded that motilin acts directly on the muscle.[3,14,15] Erythromycin and its derivatives that are thought to act through motilin receptors also contract the muscle through an action that is insensitive to block of neural transmission with TTX or atropine.[16,17] Because erythromycin and its macrolide derivative EM201 both contract rabbit duodenal muscle and displace labelled motilin from rabbit antral preparations, it has been deduced that erythromycin and motilides act at the same motilin receptor.[3] Other comparisons of contractile effects and binding also point to similarities of action of erythromycin, its motilide derivatives and motilin.[1,17,18]

Surprisingly, antagonists have not previously been used to compare the pharmacology of actions of the three classes of agonist on gut muscle, although effects on individual agonists have been reported. For example, the erythromycin analogue GM 109 antagonizes the contractile action of motilin in the rabbit small intestine[11] and the motilin analogue [phe^3,leu^{13}]motilin antagonizes both motilin- and erythromycin-induced contractions.[10]

In the present study, we have shown that motilin, erythromycin and the two motilides (ABT 229 and GM 611) all contract the longitudinal muscle of the rabbit duodenum without being affected by

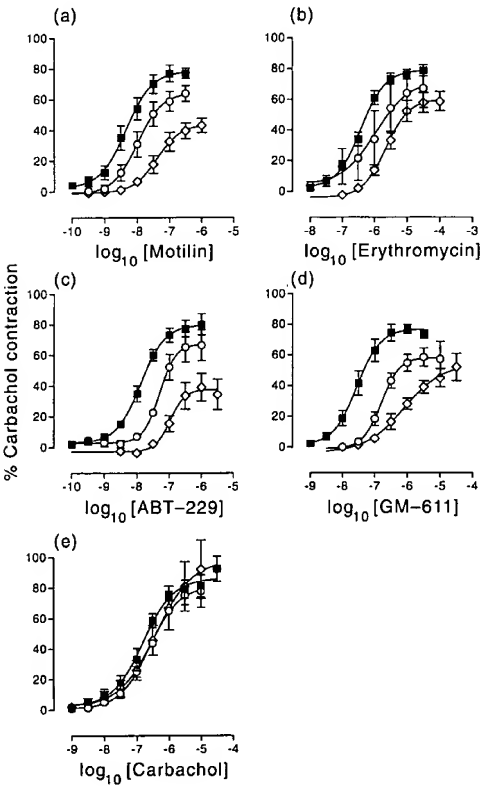

Fig. 2 Effects of the motilin receptor antagonist phe^{3}leu^{13}motilin on concentration–response curves to motilin (a), erythromycin (b), ABT-229 (c) and GM-611 (d) and the muscarinic agonist carbachol (e). Responses are in the absence (■) and presence of either 30 (○) or 300 nmol/L (◇) phe^{3}leu^{13}motilin. There were three to eight replicates for each curve.

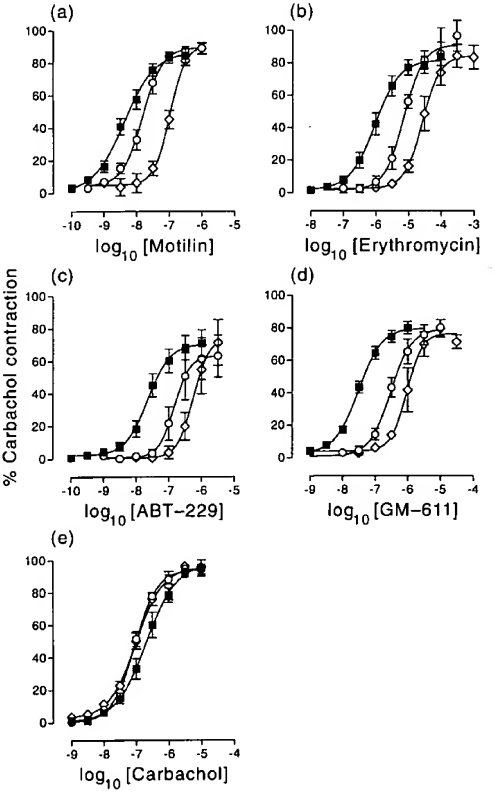

Fig. 3 Effects of the motilin receptor antagonist GM-109 on concentration–response curves to motilin (a), erythromycin (b), ABT-229 (c), GM-611 (d) and carbachol (e). Curves are in the absence (■) and presence of either 300 nmol/L (○) or 3 μmol/L (◇) GM-109. There were three to seven replicates for each curve.

TTX, which confirms that these agonists act directly on the muscle, without detectable effects on enteric neurons in this gut region. The two antagonists of motilin receptors used in the present study, [phe^3,leu^{13}]motilin and GM 109, inhibited responses to all four agonists, but did not affect the contractions caused by the muscarinic agonist carbachol. Moreover, with GM 109 as the antagonist, Schild regression analysis yielded pA_2 values in a narrow range (7.2–7.5) for all agonists. [Phe^3,leu^{13}]motilin, but not GM 109, had a partial agonist effect in these experiments despite being reported previously to have no agonist effect[10] and it depressed the maximum response to all four agonists.

Thus, the present study supports conclusions from binding-displacement studies and from more restricted pharmacological investigations that erythromycin, motilin and motilides act at the same receptor in the rabbit duodenum. The results suggest that contractile responses in this tissue are due solely to actions on intestinal muscle receptors for motilin and do not include actions on neural receptors for motilin or erythromycin, such as those that have been revealed in the rabbit antrum[19,20] and guinea-pig small intestine.[9,21] Therefore, the rabbit duodenum is a suitable tissue in which to investigate drug actions at the muscle-type motilin receptor. However, the ways in which the compounds act at this receptor may differ. [Phe^3,leu^{13}]motilin antagonizes the contractions elicited by motilin, ABT 229 and GM 611 to a greater extent than it antagonizes those of erythromycin. Moreover, GM 109 yields similar Schild plot slopes of 0.65–0.73 for motilin, ABT 229 and erythromycin, but yields a slope of 0.91 for GM 611.

In conclusion, each of these agonists appears to act at the same, motilin, receptor, but slight differences in the pharmacological profiles of the four agonists suggest that there may be several sites of agonist interaction with the receptor and/or the compounds may bind to different conformational states of the receptor.

ACKNOWLEDGEMENT

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SPECIAL REPORTS AND REVIEWS

Erythromycin and Other Macrolides as Prokinetic Agents

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Even if a scientific model, like a car, has only a few years to run before it is discarded, it serves its purpose for getting from one place to another.

—*D. Wingate*[1]

It has been known for long that antibiotics may cause gastrointestinal side effects; however, little attention has been paid to the mechanisms involved. Lees and Percy[2] found that antibiotics, able to induce colitis, depress gastrointestinal motor activity. They advanced the hypothesis that diarrhea was caused by the facilitation of bacterial proliferation by impaired intestinal motility. In a subsequent study, Percy and Christensen[3] showed that several antibiotics were able to inhibit spontaneous contractions and to reduce the tone of the distal colonic muscularis mucosae of the opossum. Erythromycin, the well-known macrolide antibiotic, was included in this study and had a similar effect as ampicillin, clindamycin, and lincomycin. In line with the earlier hypothesis it was proposed that by reducing the motility of the muscularis mucosae, the capability of microorganisms to adhere to the gut mucosa was facilitated.

However, in 1984 two groups described, independently and almost simultaneously, that erythromycin stimulated small intestinal contractile activity in the dog. Itoh et al.[4] found that erythromycin (0.03 mg/kg intravenously [IV]) induced a pattern of migrating contractions originating in the stomach and occurring at the frequency of the slow waves. The duration and migration velocity of the pattern were the same as for the spontaneous activity that occurs periodically in the fasted state, known as phase 3 activity of the migrating motor complex (MMC). Zara et al.[5] using a dose of 1 mg/kg reported similar effects, but they also noted that at higher doses (7 mg/kg IV), an immediate increase in contractile activity was seen along the whole length of the tract followed by a long-lasting disruption of the MMC.

Whereas Zara et al.[5] thought that erythromycin mimicked the effect of morphine, Itoh et al.[4] saw a closer resemblance to the effect of motilin. Motilin is a peptide discovered by J. C. Brown in 1967.[6] It is present in endocrine cells of the duodenal mucosa, from where it is released periodically in the fasted state. In humans and dogs, this release is associated with the development of phase 3 activity, and exogenous motilin induces phase 3 activity. These and several other findings suggest that motilin has a role in the hormonal regulation of the MMC (for a review see Vantrappen and Peeters[7]). However, it has also been argued that motilin release is caused by mechanical activity and that motilin does not induce phase 3 but events related to it, for example, gallbladder contractions.[8]

Because erythromycin induced a motilin release, Itoh et al.[4] suggested that it acted through motilin. Against this hypothesis was the subsequent finding that in humans erythromycin (0.25 or 1 mg/kg IV) induced phase 3 activity, but without a release of motilin.[9] We hypothetized that erythromycin could act on the motilin receptor and showed that in binding experiments erythromycin could displace motilin and that in vitro erythromycin and motilin had the same species and regional specificity.[10] These findings and earlier reports on antral motilin receptors in humans[11,12] led our group to evaluate the effect of erythromycin on gastric emptying in patients with diabetic gastroparesis. The results were clear-cut: erythromycin normalized the prolonged gastric emptying times for both liquids and solids in these patients (Figure 1).[13]

Presentation of these data at the American Gastroenterological Association meeting in Washington in 1989 proved to be so stimulating that at the next meeting in San Antonio in 1990, a whole symposium could be devoted to erythromycins's prokinetic effects. Since then interest continues to grow, and the development of more powerful erythromycin derivatives, which lack antibiotic properties, suggests that a new

Abbreviation used in this paper: MMC, migrating motor complex.

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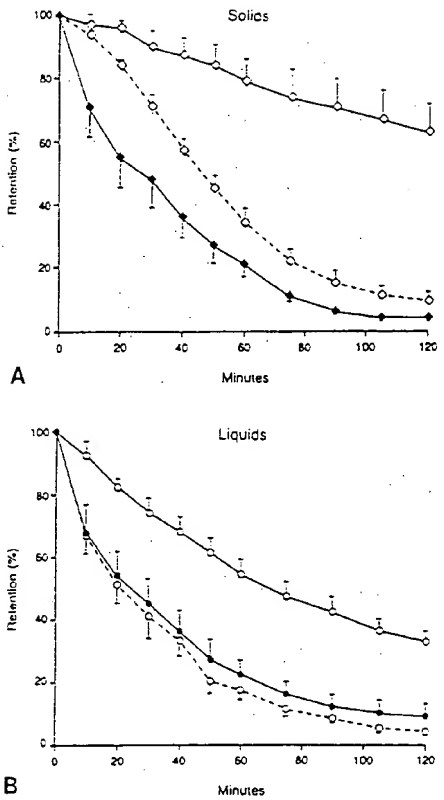

Figure 1. Rate of gastric emptying in healthy subjects and in patients with diabetic gastroparesis administered placebo or erythromycin. (A) Rate of emptying of the solid part of the test meal, expressed as the mean (±SE) percentage of isotope remaining in the stomach at various times after the ingestion of the meal, in 10 patients after IV administration of placebo (○——○) or 200 mg of erythromycin (◆——◆) and in 10 healthy subjects (○- - -○). (B) Rate of emptying of the liquid part of the test meal, expressed as the percentage of isotope remaining, in 10 patients after the administration of placebo (○——○) or erythromycin (●——●) and in 10 healthy subjects (○- - -○). (Reprinted with permission.[13])

class of powerful prokinetics has been discovered.[14] This discovery could have been made at a much earlier time. In 1960 Leaders et al.[15] noted that oleandomycin, a substance closely related to erythromycin, stimulated motor activity in the rabbit small intestine. In 1967 Benzi et al.[16] described a stimulatory effect of erythromycin on the motility of the gallbladder and the terminal bile duct. Both reports went unnoticed. Perhaps erythromycin's ability to induce phase 3 activity would also have remained just another observation if the link with motilin had not been made. It seems therefore appropriate to consider in this review erythromycin's effects on gastrointestinal motility in this perspective, even if not all motility effects of erythromycin are related to motilin.

Motilides and Motilinomimetics: In Vitro Studies

Zara et al.[5] suggested that "part of the macrolide structure might have a direct effect on smooth muscle" and that "An alteration in structure may provide a substance which would not disturb the G.I. tract without losing any of its antibiotic potency." However, while screening a number of erythromycin derivatives, Omura et al.[17] discovered a group of compounds with exactly the opposite properties: a loss of antibiotic properties but an increased ability to induce contractions. Because in vitro data showed that in the rabbit, the mechanism of action of these compounds was similar to that of motilin, Itoh and Omura[14] proposed the name motilides for all macrolides with a direct contractile effect in vitro on rabbit duodenal segments and the capacity to induce in vivo phase 3 activity in dogs. Later on evidence for interaction with the motilin receptor was obtained from binding studies and from in vitro contractility experiments.

Erythromycin displaces motilin bound to rabbit,[10,18] cat,[19] and human (T. L. Peeters, unpublished data) antral smooth muscle tissue (Figure 2). Muscle strips or intestinal segments of rat and dog do not respond to erythromycin, but in human, rabbit, and feline prepa-

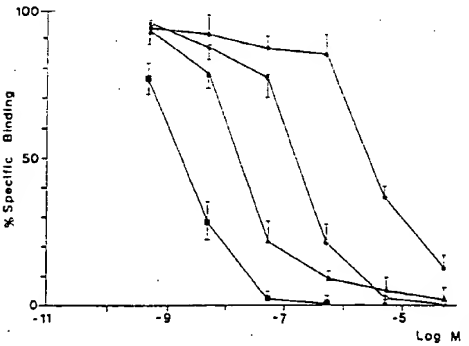

Figure 2. Displacement of iodinated Leu[13]-porcine-motilin, bound to a crude membrane preparation from rabbit duodenal smooth muscle tissue, by unlabelled Leu[13]-porcine-motilin (■), EM201 (▲), erythromycin (⊙), and erythromycin *N*-oxide (●). (Reprinted with permission.[10])

rations there is a marked increase in tone.[10,19] Moreover, in these species the response is observed with duodenal but not with ileal preparations. Apparently, erythromycin has the same regional and species specificity as motilin.[10]

In a detailed study of 73 macrolide derivatives, the potency in displacing motilin was well correlated to the potency in the tissue bath.[20] A careful study of binding and contractility was performed for the potent erythromycin derivative EM-523, synthetized by Dr. Omura (Kitasato Institute, Tokyo, Japan) and presently developed by Takeda Chemical Company (Osaka, Japan). It was shown that in binding studies using human or rabbit antral smooth muscle tissue, EM-523 competed with motilin for binding to the motilin receptor; also, in the rabbit, the aborally decreasing contractile response to motilin matched the response to EM-523.[21]

Motilides and motilin have the same mechanism of action in vitro. In the tissue bath, contractions of rabbit duodenal segments induced by motilides or motilin cannot be blocked by tetrodotoxin, atropine, or hexamethonium, suggesting a direct effect on smooth muscle cells. These contractions depend on external calcium because they are blocked by removal of calcium from the bath or calcium antagonists.[22–24] For EM-523, a detailed study showed that it depends to a similar extent on extracellular and intracellular calcium as motilin.[25] Moreover, the transduction pathway of motilin and of motilides involves inositol phosphates.[26]

These studies leave little doubt that erythromycin may act on a motilin receptor. To this may be added the existence of cross-tachyphylaxis between motilin, erythromycin, and EM-523, but not between these compounds and substance P,[23,27] and the recent discovery of the first motilin antagonist, which blocks motilin- and erythromycin-induced contractility in vitro.[28]

The lack of structural similarity between motilin and erythromycin remains puzzling. The activity of the macrolides is related to the macrolide ring, especially the configuration around C_1–C_9 (Figure 3), and to the sugars attached to it, especially the amino-*N* of the desosamine sugar.[20] In motilin, it was previously thought that both the N- and C-terminal end of the molecule were involved in receptor binding[29]; however, studies with motilin fragments showed that the bioactivity resides in the N-terminal part only.[30] From a detailed study of motilin analogues it was concluded that motilin's pharmacophore involves the residues 1 (phenylalanine), 4 (isoleucine), and 7 (tyrosine).[31] Tentatively one may assume that the two sugars and the macrolide ring each mimic one of these elements. The development of new analogues or derivatives along this line may soon prove or disprove this hypothesis.

Erythromycin can be considered a "motilinomimetic." Whether this applies to other motilides remains to be tested, but the available data suggest that this may be the case. The next question to be asked is whether all the motility effects induced in vivo by motilides are mediated via the motilin receptor. Sarna et al.[32] believe they are not. They base their conclusion mainly on in vivo effects of erythromycin that cannot be obtained with motilin. Before discussing this argument, the in vivo effects of erythromycin on gastrointestinal motility will be described and compared with those obtained with motilin.

Gastric Emptying

The initial report of erythromycin's gastrokinetic properties in diabetics[13] (Figure 1) has been confirmed by several groups, and studies have been extended to other conditions accompanied by gastroparesis. In patients with idiopathic or diabetic gastroparesis, Richards et al.[33] found an improvement after IV administration of erythromycin (6 mg/kg IV). Wadhwa et al.[34] treated a patient with diabetic gastroparesis, who was on ambulatory peritoneal dialysis because of end-stage renal disease, by adding erythromycin to the peritoneal dialysate (100 mg in 2 L). Mozwecz et al.[35] reported that a patient with postvagotomy gastroparesis, who did not respond to bethanechol or metoclopramide treatment, did respond to 250 mg erythromycin (250 mg orally, three times daily 30 minutes before the meal). With the same dosage regimen, Dull et al.[36] reported the successful treatment of a patient with progressive systemic sclerosis in which gastroparesis was the dominant feature. Xynos et al.[37] confirmed the effectiveness in postvagotomy gastroparesis and thought that it was caused by a reduction of the lag phase. Maliakkal et al.[38] described objective and subjective improvement (250 mg IV) in patients with gastroparesis induced by cancer therapy. In anorexia nervosa patients, Stacher et al.[39] showed a decrease in half-emptying time by 200 mg EM-A IV. Erythromycin is also effective in normal volunteers.[40,41]

The motor patterns responsible for the gastrokinetic effect were studied by Annese et al.[40] in normal volunteers. They found that erythromycin (200 mg IV over 20 minutes at the end of the meal) induced powerful peristaltic contractions, improved antroduodenal coordination, and induced phase 3–like patterns superimposed on the fed motility. This may explain the observation of Janssens et al.[13] that after erythromycin, the

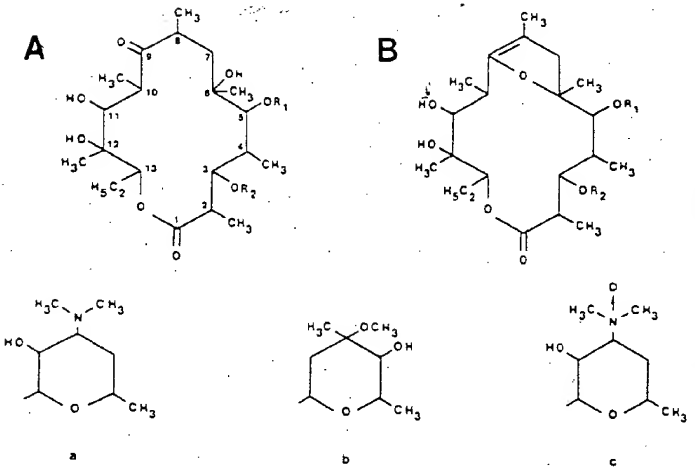

Figure 3. Chemical structures of erythromycin, EM-201, and erythromycin *N*-oxide. (*A*) The ring structure of erythromycin: in R_1 and R_2, the structures *a* (desosamine) and *b* (cladinose) are attached. In erythromycin *N*-oxide, the dimethylamino group of *a* is oxidized as shown in *c*. (*B*) The ring structure of EM-201: the same groups (R_1 and R_2) are attached to it as in *A*, i.e., *a* and *b*. Most potent macrolides have the enol configuration of this compound. (Reprinted with permission.[10])

gastric emptying rate of solids and liquids was the same. Similarly, in dogs, erythromycin causes rapid gastric emptying of untriturated solids, which also suggests that the accelerated emptying is caused by the induction of phase 3 activity.[42] The gastrokinetic effect of erythromycin therefore seems to occur at the expense of the grinding and sieving of the digesta. Fraser et al.[43] recently reported that erythromycin overcomes the retardation of gastric emptying caused by intraduodenal lipid. This effect could be ascribed to the stimulation of antral and duodenal pressure waves and to an inhibition of pyloric pressure waves.

A question that should be considered is whether the gastrokinetic properties of erythromycin are shared by motilin. It must be said that earlier studies led to somewhat disappointing results. Depending on the species and the type of meal, enhancement,[44,45] lack of effect,[46] and even inhibition[47] of gastric emptying have been reported. These studies and the clear-cut relationship of motilin with the MMC have led to the general consensus that the effect of the peptide is limited to the fasted state. The findings with erythromycin prompted two groups to investigate motilin's postprandial effect in patients with diabetic gastroparesis. Allescher et al.,[48] using a dose of only 0.2 pmol · kg^{-1} · min^{-1}, found normalization during the first 15 minutes after the meal. We used a dose of 10 pmol · kg^{-1} · min^{-1} over 90 minutes and found a normalization of emptying.[49] However, others failed to detect an effect of motilin (3.7 pmol · kg^{-1} · min^{-1}) on postprandial motor patterns in humans, although erythromycin (200 mg IV) had an effect.[50] However, in dogs the ability of pharmacological doses of motilin (222 pmol/kg) to induce contractions in the fed state has been shown by Iroh[51]; also, Carlson et al.[52] showed that erythromycin (1 mg/kg IV for 1 hour) as well as motilin (500 mg/kg IV for 1 hour) enhanced delayed gastric emptying in dogs after Roux-Y antrectomy.

Erythromycin's effect in dogs with vagotomy and Roux-Y antrectomy was blocked by atropine.[52] Clearly this cholinergic mechanism does not involve the vagus, as can also be derived from the effectiveness of erythromycin in postvagotomy patients and in patients with diabetic gastroparesis (who presumably have, to some degree, autovagotomy). In vitro, motilin acts directly on human smooth muscle but is ineffective on canine smooth muscle. The in vivo mechanism of motilin's gastrokinetic effect is unknown, but at least there are no data contradicting the hypothesis that it is the same as erythromycin's. If one accepts that both substances owe their gastrokinetic properties to their ability to induce phase 3–like patterns, the issue should also be seen in the light of their mechanism of action in the fasted state, which will be discussed later on.

Esophageal Motility

It is fitting that the demonstration of erythromycin's gastrokinetic and motilinomimetic properties led to a study of its effect on the lower esophageal sphincter (LES). Indeed, some of the earliest investigations on the biological role of motilin concerned the lower esophagus. Thus the ability of motilin, given IV, to induce contractions in the human, canine, and opossum lower esophagus was already shown in the

mid 70s.[53–56] In the three species the effect is mediated by cholinergic nerves, because it is reduced in vivo[53–56] and in vitro[57,58] by atropine and hexamethonium. When the relation of plasma motilin levels with the duodenal MMC was discovered, the topic was abandoned; however, later on it was found that the MMC had an esophageal component.

Three groups have now shown that erythromycin increases the LES pressure in the fasted[59,60] and in the fed state.[61] Only one group[59] found also an effect on duration and progression velocity of esophageal contractions, but the three groups agree that their amplitude is unaffected. It should be noted that Chaussade et al.[59] did not administer erythromycin (150 mg IV) at a specific point of the MMC cycle. This may induce an additional variability because LES pressure increases towards phase 3. Janssens et al.[60] avoided this problem by giving erythromycin (3.5 mg/kg IV) after passage of a phase 3, and Dalton et al.[61] did their study (500 mg orally) in the fed state "because of the wide variation of LESP in the interdigestive state." If the effect on the LES is related to the induction of the MMC, then the dose used in these studies was perhaps too high. As will be discussed below, a dose of 40 mg IV is enough to induce phase 3 activity in humans and 200 mg IV is already too much.[62] Future studies should take this into account.

The effects of erythromycin on esophageal motility and on gastric emptying indicate that it may be useful in the treatment of gastroesophageal reflux. However, the first two studies tackling this issue gave basically negative results.[63,64] Interestingly, the gastroesophageal reflux caused by white wine was reversed by infusing 3.5 mg/kg EM-A before ingestion.[65]

Colonic Motility

Little attention has been paid to motilin's effect on colonic smooth muscle. Strunz et al.[66] noted that motilin induced contractions in the circular colonic muscle strips of the rabbit, but not in the taenia coli. In humans the situation was reversed. Adachi et al.[67] reported that segments of the rectum of the rabbit oriented in the longitudinal axis responded to motilin, but the response was only 46% of the maximal response to acetylcholine compared with 102% in the duodenum. In vivo it was found that exogenous motilin stimulated motor activity in the human colon[68] and induced colonic motor complexes in the canine colon.[69] Itoh[51] also noted that in dogs a large dose of motilin induced colonic motor activity, sometimes defecation. Recently[70] we showed a high density of motilin receptors in the rabbit colon by binding studies and a contractile response that was most pronounced in circular muscle strips (80% of the response to acetylcholine vs. 20% for longitudinal strips). Isolated myocytes from the rabbit colon also respond to motilin and to erythromycin.[71] A rationale for a study of erythromycin's effect on colonic motility may be derived from these findings.

Zara et al.[5] found that in dogs IV erythromycin first induced a period of intense colonic motor activity that was followed by a period of inhibition. A prokinetic effect of erythromycin (200 mg orally, four times daily) on the human colon was reported by Hasler et al.,[72] but erythromycin (500 mg IV over 1 hour) was ineffective in constipated patients.[73] Bradette et al.[74] compared the effect of motilin (100 ng/kg over 10 minutes IV) with that of erythromycin (200 mg over 20 minutes IV) on different regions of the human colon in fasting subjects and after a meal. Only in fasting subjects did both substances provoke an increase in contractile activity, and this effect was limited to the sigmoid colon. On the other hand, three other studies failed to detect any effect.[75–77]

Biliary Tract

Bile flow depends on hepatic secretion, gallbladder contraction, and sphincter of Oddi motility. Motilin's effect on hepatic secretion has not been studied. For the gallbladder it depends on the species, but in all species studied to date, motilin increased sphincter of Oddi motility.

Gallbladder

In vivo, motilin stimulates gallbladder contraction in dogs,[78,79] increases gallbladder pressure in pigs,[80] but is without effect in humans[81] and in cats.[82] In vitro, motilin has been found to be without effect on gallbladder smooth muscle strips from humans or rabbits.[66,83]

Given the absence of an effect of motilin on gallbladder emptying in humans, the finding that erythromycin stimulates gallbladder contraction in normal volunteers and in postcholecystolithotomy patients[84] comes as a surprise. However, these data have been confirmed[85] and extended to patients with diabetic autonomic neuropathy.[86] These patients have an increased fasting gallbladder volume, which was reduced by a single oral dose of erythromycin (500 or 1000 mg). Itoh et al.[87] induced gallbladder contractions in humans with low doses of EM-523 (0.5–2.0 mg IV). As was noted in the introduction, the ability of erythromycin to induce gallbladder contractions was already described in 1967.[16] In view of all these data, the effect

of motilin on the human gallbladder should be reexamined, because it has recently been reported that isolated cells from human gallbladder respond to motilin.[88]

In dogs, observations made with motilin run parallel to those made with motilides. Thus motilin and EM-523 (1–10 μg/kg IV) induce gallbladder contractions, and their effect is blocked by atropine, hexamethonium, dopamine, and 5-hydroxytryptamine antagonists.[89] A recent study suggested that cholecystokinin is involved in motilin-induced gallbladder motility.[90] However, motilin's effect cannot be blocked with the cholecystokinin antagonist L-364718 in dogs,[91] and loxiglumide is without effect on erythromycin-induced gallbladder contraction in humans.[92] According to Fiorucci et al.[85] erythromycin's effect could be mediated by a motilin release. They could block erythromycin's effect (and motilin release) by atropine and by somatostatin.

Sphincter of Oddi

Motilin has been shown to increase myoelectric activity in the sphincter of Oddi of the opossum,[93] the cat,[82] and the rabbit.[94] Intraluminal pressure waves were recorded in the dog. Their amplitude and frequency were increased by motilin.[95] This increased motility decreases transphincteric bile flow, as was shown in the dog[96] and the cat.[82]

Baker et al.[97] compared the effect of EM-A and motilin on circular and longitudinal smooth muscle strips of the sphincter of Oddi of the opossum. They found that both had a direct effect, with motilin being about 1000-fold more potent than erythromycin. The effect was diminished by verapamil and abolished in calcium-free solution. These findings are very similar to those reported for the rabbit duodenum.[10] Saccone et al.[98] verified that motilin and erythromycin increased phasic contraction frequency and decreased transphincteric flow in the Australian possum. By manometry, an increase of amplitude and duration of phasic contractions was also shown in humans.[99]

MMC

We may now return to the effects on fasting small intestinal motility, which were already mentioned in the introduction and which were at the origin of the present interest in motilin and erythromycin.

Further explorations of the observations of Itoh et al.[4] and Zara et al.[5] confirmed the importance of the dose. Thus, Otterson and Sarna[100] noted that a low dose of erythromycin (1 mg/kg IV) induces phase 3 activity but higher doses reduce (10 mg/kg IV) or increase (25 mg/kg IV) the length of the MMC cycle. High doses also increased the incidence of giant migrating contractions, retrograde giant contractions, and periods of amyogenesia. The effect of a higher dose (10 mg/kg IV) was also studied by Holle et al.[101] who described a sudden and marked increase of contractile activity occurring simultaneously along the whole length of the small bowel after erythromycin. These investigators also observed retrograde giant contractions, retching, and vomiting, but no amyogenesia.

In humans, in contrast to the dog, higher doses of erythromycin seem to affect the antrum only, and the small bowel tends to be inhibited (Figure 4). Sarna et al.[32] reported that a therapeutic dose (500 mg) did not induce an MMC or a motilin release, but stimulated antral contractile activity. Tack et al.[62] compared the effect of three doses (40, 200, and 350 mg) and concluded that the lowest dose induced an MMC, whereas a prolonged period of antral hypermotility was seen after 200 and 350 mg. Chen et al.[102] studied the effect of erythromycin on gastric myoelectric activity by cutaneous recording. They concluded that erythromycin (6 mg/kg) reduced gastric slow wave frequency.

The mechanism by which erythromycin induces phase 3 activity has not been fully elucidated, but all present observations in the dog model run parallel to those made with motilin. Like motilin, erythromycin induces a true phase 3 (followed by phase 1) only in the fasted state and this effect is inhibited by feeding and by gastrin.[4] Erythromycin's effect on the MMC is blocked by anticholinergic agents,[102] but so is motilin's.[51] Perhaps most intriguing is the observation that 5-hydroxytryptamine antagonists only block motilin's effect on gastric phase 3 activity[104] and that this applies also to motilides.[87] The early suggestion that erythromycin's effect is caused by a release of motilin can be discarded. Although a release of motilin following erythromycin has been reported in dogs,[4] in humans most studies failed to detect such an increase.[9,62,105]

Side effects have not been noted during motilin infusion, and the induction of an MMC is not accompanied by cramps. Therefore, it seems most likely that the side effects of erythromycin are not caused by the induction of an MMC but by the abnormal patterns described by Otterson and Sarna.[100] If one aims at a beneficial effect, it is clear that low doses must used. A few attempts have been made in this direction in chronic idiopathic pseudo-obstruction and in postoperative ileus.

Ps ud - bstruction and Ileus

Miller et al.[106] studied the effect of erythromycin on interdigestive motility in six controls and three

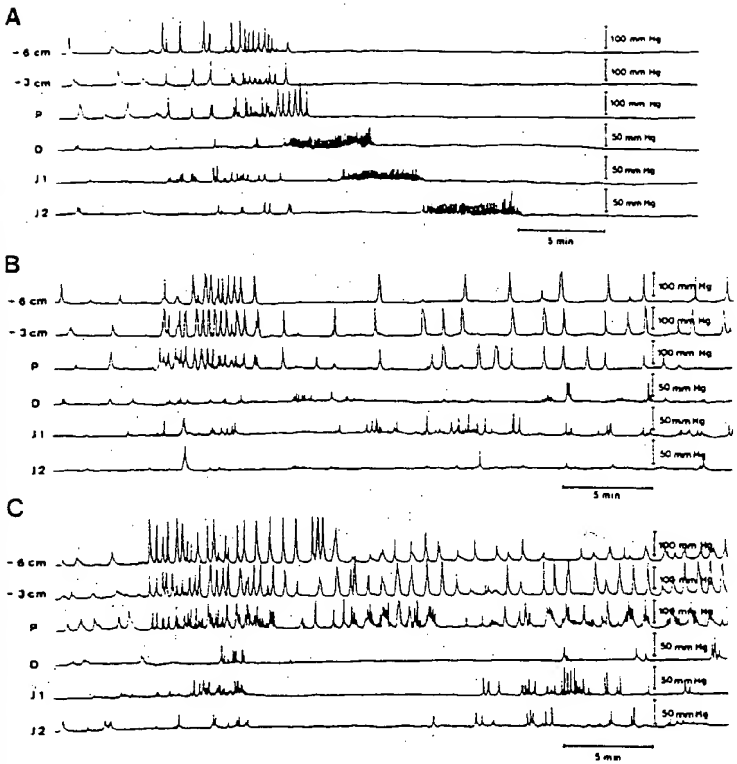

Figure 4. Effect of IV erythromycin on the upper gastrointestinal motility in healthy volunteers. All traces begin at the start of the infusion. (*A*) Erythromycin, 40 mg IV, caused a premature MMC that originated in the stomach. (*B*) Erythromycin, 200 mg IV, caused a burst of phase 3–like contractile activity in the antrum. This burst of phase 3–like antral contractions did not migrate to the small intestine and was not followed by a phase 1. Instead, a prolonged period of strong antral contractions was seen. (*C*) A similar response was observed after administration of 350 mg erythromycin. −3 cm and −6 cm, 3 and 6 above prepyloric level. (Reprinted with permission.[82])

patients with chronic idiopathic pseudo-obstruction. They infused 3.5 mg/kg erythromycin IV for 20 minutes after phase 3 and reported a rhythmic burst of antral contractions migrating to the duodenum in two of six controls and in three of three patients. Improvement of clinical symptoms in one patient was reported by Berger et al.[107] Chami et al.[108] studied six patients. In all patients, IV EM (40 mg) induced antral phase 3, which migrated into the small intestine in five patients. With daily oral administration (40 mg, three times a day) symptoms improved, but two patients stopped the therapy because of abdominal cramps. The same group reported an improvement of symptoms in a patient with dysmotility associated with scleroderma.[109]

In a group of children with chronic pseudo-obstruction, erythromycin (3 mg/kg over 1 hour IV) induced phase 3 activity in those who also had phase 3 during control conditions. It was much less effective in others.[110] In children with the presumptive diagnosis of chronic intestinal pseudo-obstruction, it has also been described that erythromycin (3 mg/kg IV over 1 hour) facilitated the postpyloric passage of tubes during duodenal intubation for antroduodenal manometry.[111] The investigators assume that propagating antral contractions sweep the tube through the open pyloric channel into the duodenum.

Whereas motilin has never been used to treat idiopathic pseudo-obstruction, one attempt was made to treat postoperative ileus. However, Ruppin et al.[112] saw no beneficial effect of motilin 1 or 2 days after cholecystectomy. More recently, the potential of motilides was investigated. Holle and Forth[113] reported an increase of the motility index induced by erythromycin in dogs, but Bonacini et al.[114] found that treatment of cholecystectomy patients with erythromycin did not affect the appearance of flatus or bowel movements. In contrast, Hanyu et al.[115] reported a dose-dependent decrease of the time until the start of bowel sounds in a similar patient population but using EM-523.

Mechanism of Action in Vivo

The preceeding survey of the motility effects of erythromycin in vivo (see also Table 1), written with the effects of motilin as the background, indicate that the effects that are best documented, i.e., induction of the MMC and acceleration of gastric emptying, can be obtained with both. With respect to the actions on the esophagus, colon, and biliary tract, the situation is less clear but not directly contradictory. The most important differences between the action spectrum of both compounds are the abnormal patterns induced by high doses of erythromycin in the fasted state. However, one should keep in mind that few experiments have been performed with large doses of motilin; also, because of the different pharmacokinetic properties of erythromycin and motilin (plasma half-lifes of 90 and 4 minutes, respectively), it is difficult to mimic a large dose of erythromycin with motilin. Nevertheless, a few studies have been reported in the literature, showing that long-term administration of motilin (0.6 $\mu g \cdot kg^{-1} \cdot h^{-1}$ for 4 hours) induces amyogenesia[116] and that large boluses (3.0 μg/kg) induce vomiting.[51] At high doses, the action spectrum may differ less than suggested by the literature. Even if some differences remain, they do not disprove that erythromycin is a motilin agonist.

Erythromycin is a large molecule, and different parts of it may have different actions, some of them emerging only at high concentrations. With all the interest into its prokinetic effects, one should not forget that the substance is used as an antibiotic. In the motilides, these two properties can clearly be separated, because some erythromycin derivatives with increased motor activity are devoid of antibacterial activity. Recently Minocha and Galligan[117] presented evidence that erythromycin may inhibit contractions of the guinea pig small intestine by a direct effect on enteric neurons. This effect was not observed with motilin, and it could be the basis of inhibitory in vivo responses. The concentrations required for this effect are rather high. The half-maximal response was at 161 μmol/L, which is about 150 times more than for the contractile effect on the rabbit duodenum. The authors suggested that erythromycin may inhibit Ca^{2+} entry. Also, a similar mechanism had also been evoked by Lees and Percy[2] to explain the inhibitory effect of antibiotics on guinea pig ileum contractility, because they could reverse it by increasing the Ca^{2+} concentration. Percy and Christensen[3] noted that the inhibitory effect of antibiotics on the colonic muscularis mucosae is related to lipophilicity. It is therefore possible that it is caused by a nonspecific effect on membrane cation permeability, in which case all excitable tissues may be affected. Indeed, it has been reported that high concentrations of erythromycin (10^{-3} to 10^{-4} mol/L) induce skeletal muscle paralysis,[118] ventricular tachyarrhythmias,[119,120] and spasm of uterine smooth muscle.[121] Some of these effects may perhaps also be obtained with high concentrations of motilin (motilin has a hydrophobic N terminus), but it is unlikely that they are mediated by a motilin receptor.

Do low doses of erythromycin induce the MMC through motilin receptors? The fact that the in vitro contractile response does not bear any similarity with the MMC has been used as an argument against such a mechanism[32]; however, this applies not only to erythromycin but also to motilin. Therefore, this argument leads to the conclusion that motilin itself . . . does not act through motilin receptors! This may very well be the case in the sense that the muscular motilin receptor, characterized in rabbit tissue by in vitro contractility and by binding studies, is not identical with the receptor responsible for the induction of the MMC and which is, in humans and in dogs, neuronal. However, the neuronal and the muscular motilin receptors seem to be well related to each other, because the ability of a substance to interact with one can be predicted from its ability to interact with the other. This at least is one way to interpret the remarkable correlation between the potency of motilides in vitro (in binding studies using rabbit tissue) with their potency in vivo (in another species, dogs!). Thus, oleandomycin, which is much less potent in vivo than erythromycin,[122] and EM-523, which is much more potent,[123] have corresponding potency rankings in binding studies. The same is true for azithromycin, which has a stronger effect, and clarithromycin, which has a weaker effect than erythromycin.[124] For a series of eight erythromycin derivatives, the in vivo contractile effect in dog was almost perfectly correlated ($r = 0.93$) with the ability to displace motilin (H. N. Nellans, Abbott, Chicago, IL; personal communication, May 1992). Compounds without effect further support these correlations. Thus, the 16-membered macrolides josamycin, tylosin, kitasamycin,[20] and midecamycin (T. L. Peeters, unpublished data) have no effect in binding studies and are ineffective in vivo.[122,125–127] Ineffective in vivo and in binding studies are also 14-numbered macrolides lacking sugars attached to the ring.[20,126]

Recently Tack et al.[128] showed that motilin and erythromycin act on the same subpopulation of myenteric neurons in the guinea pig antrum, a finding that

Table 1. Effects of Motilides on the Human Gastrointestinal Tract

Organ and function	Subjects	Dose[1]	Time[2]	Drug[3]	Effect	Study
Esophagus, LES	Volunteers	150 mg IV, 20 min	Fasting	EM	Increases LES pressure and duration of peristaltic contractions	Chaussade et al.[59]
	Volunteers	3.5 mg/kg IV	Phase 3, + 5 min	EM	Increases LES pressure	Janssens et al.[60]
	Volunteers	500 mg PO	pp	EM	Increases LES pressure	Dalton et al.[61]
	Patients with reflux	250 mg PO	qid	EM	Tendency to reduce long reflux episodes	Champion et al.[63]
	Patients with reflux	250 mg PO	tid	EM	No effect on reflux	Harrison et al.[64]
	Volunteers	3.5 mg/kg IV	pp	EM	Suppresses reflux caused by white wine	Pfeiffer et al.[65]
Stomach, gastric emptying	Diabetics[a]	200 mg IV, 20 min	pp	EM	Accelerates empyting of liquids and solids	Janssens et al.[13]
	Diabetics[a]	250 mg PO	Meal, −20 min, t.i.d.	EM	Accelerates emptying of liquids and solids (less pronounced)	Janssens et al.[13]
	Diabetics, idiopathic gastroparesis	6 mg/kg IV	pp	EM	Accelerates gastric empyting	Richards et al.[33]
	Diabetics[a]	100 mg IP	Meal, −30 min, t.i.d.	EM	Normalizes gastric empyting, suppresses vomiting	Wadhwa et al.[34]
	Postvagotomy gastroparesis	250 mg PO	pp, t.i.d.	EM	Normalizes gastric empyting	Mozwecz et al.[35]
	Systemic sclerosis[a]	250 mg PO	pp, t.i.d.	EM	Improves gastric empyting	Dull et al.[36]
	Cancer therapy[a]	250 mg IV	pp	EM	Improves gastric empyting, symptoms	Maliakkal et al.[38]
	Anorexia nervosa[a]	200 mg IV	pp	EM	Accelerates gastric empyting	Stacher et al.[39]
	Volunteers	200 mg IV, 20 min	pp	EM	Accelerates gastric empyting by improving antroduodenal coordination	Annese et al.[40]
	Volunteers	2–3 mg/kg IV, 15 min	pp	EM	Accelerates gastric empyting	Keshavarzian and Isaac[41]
	Volunteers	3 mg/kg IV, 15 min	Lipid, +20 min	EM	Overcomes the effect of intraduodenal lipid	Fraser et al.[43]
MMC	Volunteers	1–3 mg · kg^{-1} · h^{-1} IV, 15 min	Fasting	EM	Induces MMC	Tomomasa et al.[9]
	Volunteers	40 mg IV	Fasting	EM	Induces MMC	Tack et al.[52]
	Volunteers	200 and 350 mg IV	Fasting	EM	Induces contractions in the stomach	Tack et al.[52]
	Volunteers	500 mg IV, 15 min	Fasting	EM	Induces gastric contractions, small intestine innibited	Sarna et al.[32]
	Volunteers	6 mg/kg IV	Fasting	EM	Disturbs gastric slow wave frequency	Chen et al.[102]
	Pseudo-obstruction	3.5 mg/kg IV	Phase 3, +20 min	EM	Induces phase 3, see also references 101 and 102	Miller et al.[106]
	Ileus patients	250 mg IV	Fasting	EM	Ileus not affected	Bonacini et al.[114]
	Ileus patients	0.25–4 mg IV	Fasting	EM-523	Reduces duration of ileus	Hanyu et al.[115]
Gallbladder	Volunteers	500 mg PO	Fasting	EM	Reduces gallbladder volume	Catnach et al.[84]
	Volunteers	500 mg PO	pp	EM	Increases gallbladder emptying rate	Catnach et al.[84]
	Gallstone patients	500 mg PO	pp	EM	Increases gallbladder emptying rate	Catnach et al.[84]
	Volunteers	50–100 mg/h IV	Fasting	EM	Reduces gallbladder volume	Fiorucci et al.[85]
	Volunteers	0.5–2.0 mg IV	Fasting	EM-523	Induces gallbladder contraction	Itoh et al.[87]

(continued on following page)

further supports this concept. Neuronal motilin receptors should be further characterized, but now that a motilin antagonist is available, the involvement of motilin receptors of all erythromycin-induced effects can be investigated in vivo. Obviously the MMC will be on top of the list, but given the close similarity between effect and pharmacology of motilin- and erythromycin-induced contractions of the canine gallbladder outlined above, this issue will certainly be studied soon.

Table 1 (continued). Effects of Motilides on the Human Gastrointestinal Tract

Organ and function	Subjects	Dose[1]	Time[2]	Drug[3]	Effect	Study
Sphincter of Oddi	Volunteers	200 mg IV	Fasting	EM	Increases amplitude and duration of phasic contractions	Beker et al.[69]
Colon	Volunteers	200 mg IV, 20 min	Fasting	EM	Induces contractions in the sigmoid region only	Bradette et al.[74]
	Patients, constipation	500 mg IV, 1 h	Fasting	EM	No effect on colonic motility, nausea	Bassotti et al.[73]
	Patients, IBS	500 mg IV, 30 min	Fasting	EM	No effect on colonic motility, nausea	Delvaux et al.[75]
	Volunteers	6 mg/kg	Fasting	EM	No effect on colonic motility, nausea	Yeaton et al.[76]
	Volunteers	500 mg PO, or 1.8 mg/kg IV	Fasting	EM	No effect on sigmoid motility or colonic transit	Jameson et al.[77]

NOTE. Times are the period over which the drug is administered IV, when this information was given by the authors. Note that erythromycin should not be given as a bolus, because this is rather painful.
PO, orally; IP, intraperitoneally; t.i.d., three times a day; q.i.d., four times a day; pp, postprandial.
*Patients with gastroparesis.

Conclusions and Perspectives

It is now well established that erythromycin has profound effects on gastrointestinal motility. Some of these effects are mediated through motilin receptors, and some can be exploited therapeutically. One may suggest that only beneficial effects are mediated through motilin receptors.

The most convincing data (in patients with gastroparesis) have been obtained when erythromycin was administered IV. The efficacy of oral therapy may be less because of the conditions of such patients, and the timing of the administration may be crucial. In a patient with postvagotomy gastroparesis,[35] the drug was effective when administered 30 minutes, but not 120 minutes, before meals. Long-term treatment may pose another problem. Because erythromycin is able to down-regulate the motilin receptor in rabbit,[129] desensitization may develop. However, Janssens et al.[13] found that the drug was still effective in diabetics after 4 weeks oral therapy; also, 1-week administration did not compromise the gastrokinetic response in a dog model.[130] More extensive studies over longer periods are needed to rule out the development of tachyphylaxis, but it may be noted that Wadhwa et al.[34] treated a patient during 6 months with erythromycin administered intraperitoneally. During this period the patient, with end-stage renal disease secondary to diabetic nephropathy and with symptoms of nausea and vomiting, remained symptom free.

The most promising area of application appears to be in the treatment of gastroparesis, but motilinomimetics may also be useful in patients with a risk of gallstone formation as a prophylactic to reduce recurrence or to aid fragment clearance after extracorporeal shock-wave lithotrypsy.[84] Given the limited number of therapeutic agents able to help patients with pseudo-obstruction, the potential of erythromycin in treating this condition should be further explored. The usefullness in gastroesophageal reflux should not be discounted too quickly. Trials in gastritis, duodenal ulcer, small intestinal bacterial overgrowth, postoperative ileus, and constipation should be considered.

Is erythromycin the prokinetic drug of the future? The question was raised rhetorically, and somewhat skeptically.[131] The comment provided at least one answer: it certainly is a drug available *today*. Different routes of administration, dosage regimens, and indications can all be explored. These data will pave the way for the introduction of more potent derivatives devoid of antibacterial activity and with perhaps different pharmacokinetic properties. Comparisons should also be made with other prokinetic drugs. Inatomi et al.[123] compared the effects of the derivative EM-523 with those of motilin, cisapride, metoclopramide, and trimebutine. The threshold dose for EM-523 was 100 times higher than for motilin but 100 times lower than for cisapride. Clearly, "motilides" are promising as prokinetic drug, but the same could be said of motilin itself. Motilin analogues may therefore form an alternative for motilides.

In trials using erythromycin, the risk of inducing resistant bacterial strains should be weighed against the benefit of the treatment. Certainly motilides without antibacterial properties such as EM-523, or the recently announced analogue EM-574,[132] will be most welcome. Careful consideration should also be given

to the dosage regimen. Because of the low doses required for the prokinetic effect, other side effects of high doses of erythromycin, for example ventricular tachycardia, are unlikely to occur, although it may be safe in children to monitor the electrocardiogram during infusion.[133] One should also be aware of the fact that not all of the prokinetic effects may be beneficial. The possibility that erythromycin causes pyloric stenosis[134,135] should be reexamined in light of the present findings. On the other hand, the concern that erythromycin during pregnancy could lead to the passage of antenatal meconium,[136] an event that seems to be related with high motilin levels, is not supported by the clinical findings.[137]

The similarity of erythromycin's effect with that of motilin and the interaction with the motilin receptor are at the origin of the present interest in the motilides. While clinical trials explore applications, basic studies should seek to explain further how motilin and erythromycin are related. As yet the motilin agonist theory has been rather useful. It led intuitively to the discovery of erythromycin's gastrokinetic properties and provides an objective model to predict the prokinetic potential of erythromycin derivatives. Interaction with the motilin receptor may also serve to predict whether a antibacterial derivative will have undesirable side effects. Now that a motilin antagonist is available, it will be possible to check whether these assumptions and models are correct. It is clear that this will lead to new insights into the regulation of gastrointestinal motility in general and motilin's role in particular. If along the road the motilin agonist theory should have to be partially discarded, then it is beyond any doubt that the new model can only be more exciting.

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communications to the editor

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Why Not To Use Erythromycin in GI Motility

To the Editor:

In their excellent review of GI complications in patients receiving mechanical ventilation, Mutlu and colleagues[1] discuss the beneficial effects of erythromycin at a daily dose of 200 mg, plus metroclopramide and cisapride on GI motility.

Even if GI hypomotility is a serious problem in patients admitted to the ICU, its clinical impact seems to be much less important than nosocomial infections of the respiratory tract, which may develop in up to 20% of patients who have received mechanical ventilation for a period > 48 h.[2]

The use of erythromycin, at doses far below the concentrations necessary for an inhibitory effect on susceptible bacteria, provides close to ideal conditions for the induction of bacterial mutation and selection. Since there are at least two other effective nonantibiotic drugs to enhance GI motility, it seems reasonable to use one of these in the first line of treatment rather than erythromycin, which has prokinetic properties only as a side effect. To our knowledge, there is no study addressing the question of the resistance of fecal bacteria populations before and after the use of erythromycin at subinhibitory concentrations. However, emergence of bacteria increasingly resistant to macrolide antibiotics has recently been reported.[3]

In the absence of reliable data, the use of erythromycin *just* for its prokinetic effects should thus be avoided, and its prescription should be reserved for infections due to susceptible bacteria. Only an approach toward the use of erythromycin as reasonable as our approach toward other antibiotics may help to restrain the emergence of resistant populations.

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3 Gay K, Baughan W, Miller J, et al. The emergence of *Streptococcus pneumoniae* resistant to macrolide antimicrobial agents: a 6-year population-based assessment. J Infect Dis 2000; 182:1417–1424

To the Editor:

Guerin and Leibinger raise an insightful point about the use of erythromycin for GI hypomotility in patients receiving mechanical ventilation. Sublethal concentrations of antibiotics exert selective pressure on bacteria and can contribute to the development of resistance.[1,2] While concerns regarding the development of antimicrobial resistance are, in general, relevant, we are unaware, as Drs. Guerin and Leibinger have also pointed out, of any data to support the clinical relevance of this hypothesis regarding a short course of low-dose erythromycin.

GI hypomotility affects up to 50% of patients receiving mechanical ventilation, it is associated with significant complications (aspiration, esophagitis), and it impedes the delivery of enteral nutrition. Furthermore, hypomotility may contribute to overgrowth and translocation of bacteria across the bowel wall, which can be a cause of spontaneous bacterial peritonitis and a contributor to multiorgan system failure. Parenteral nutrition as an alternative for enteral route in intractable cases of GI hypomotility is associated with myriad complications (*ie*, catheter infections, deep venous thrombosis). Therefore, GI hypomotility is a significant problem that should be treated if possible. Unfortunately, treatment options are limited; cisapride is no longer available in North America, and metoclopramide does not always work. Thus, short-term use of low-dose erythromycin is a reasonable approach to promote GI motility.

Until new enterokinetic drugs such as 5-HT4 receptor agonists (*ie*, prucalopride)[3,4] become available, and given the ramifications of hypomotility in critically ill patients, we believe that the benefits of a short-course treatment with once daily low-dose erythromycin for intractable GI hypomotility outweigh the unproven risk of erythromycin-induced bacterial resistance.

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Tumor Markers for Diagnosing Malignant Pleural Effusion?

To the Editor:

In the April 2001 issue of *CHEST*, Paganuzzi and colleagues[1] analyzed two tumor markers (carcinoembryonic antigen [CEA], and CYFRA 21–1) in pleural fluid and concluded that elevated levels of these tumor markers suggest a diagnosis of malignant pleural effusion, and in patients with poor clinical conditions, diagnosis should be made on the basis of tumor markers alone. Can this conclusion be obtained from their results? I do not think so. In this series, CEA and CYFRA 21–1 sensitivity was 31% and 78%, respectively, and specificity was 91% and 80%. The possibility that a patient has a malignant pleural effusion if the tumor marker is "positive" depends on the pretest probability (prevalence). In that study, performed at least in part in a cancer center, the prevalence of malignancy was 68%. However, the prevalence in general hospitals is, by far, lower.[2–4] For instance, in an epidemiologic study,[2] the prevalence of effusions associated with malignancy was 24%, and in 273 consecutive patients studied by our group in a department of internal medicine,[4] the prevalence was 33%. Because of the fact that in about half of the patients with malignant pleural effusion the first cytologic study finding is positive (consequently, tumor markers are not necessary), the true prevalence (effusions with a negative cytologic study finding) is still lower. In Table 1, the posttest possibility of malignancy for several percentages of prevalence and for both tumor markers has been calculated. As can be observed, with a high prevalence the result should be considered somewhat suggestive but not diagnostic of malignancy (probability 77% for CEA, and 60% for CYFRA 21–1), and with the actual pretest probabilities in general clinical practice, the probability is purely by chance (for example, with prevalence 20% and a "positive" test finding, the probability of malignancy is 46% and 49%, respectively). Moreover, clinical data are, frequently, enough for suspecting malignancy, and in patients with pleural effusion of unknown cause malignancy is not frequent.[5] Whether tumor markers are useful in this subgroup of patients (undiagnosed and without clinical suspicion of a malignant cause) needs to be demonstrated. In conclusion, tumor markers are not useful for diagnosing a malignant pleural effusion, and is a test necessary for suggesting malignancy when, in most cases, it is clinically easy to suspect?

Table 1—*Probability of Malignant Pleural Effusion When the Tumor Marker Is Positive Depending on the Prevalence of Malignancy**

Prevalence, %	Tumor Marker, %	
	CEA	CYFRA 21-1
50	77	80
40	69	72
30	60	63
20	46	49
15	38	41

*Cutoff values, sensitivity and specificity of CEA and CYFRA 21-1 reported by Paganuzzi et al.[1]

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To the Editor:

The data published in *CHEST*, April 2001, were focused on mesothelioma. We and other authors believe that tumor markers may be especially useful in mesothelioma patients with poor clinical conditions, when cytology is negative or uncertain and when the performance status does not suggest a more aggressive approach.

We did not say, as reported by Pachon, that "elevated levels of CEA and CYFRA 21-1 suggest a diagnosis of malignant pleural effusions," but we said that "high CYFRA 21-1 levels with low CEA levels are suggestive of mesothelioma, whereas high levels of CEA alone or of both markers can show evidence of malignant pleural effusion, excluding mesothelioma." In our study, CYFRA 21-1 sensitivity in 19 cases of mesothelioma with negative or uncertain cytology was 89%, with no cases showing CEA values higher than the cut-off level.

Furthermore, we think that the prevalence of effusions associated with malignancy is related to different factors (incidence of tuberculosis, pollution exposure, features of the populations, and so on). For instance, in our geographic area there is intensive occupational and environmental exposure to asbestos and to other carcinogenic substances. Consequently, the incidence of malignant pleural effusion is greater than in other areas, like the region of Central Bohemia cited by Pachon.

We don't agree with the opinion of Dr. Pachon that "clinical data are, frequently, enough for suspecting malignancy." In our experience, despite conditions of general well-being, some patients developed pleural effusion with clinical and biochemical characteristics of a parapneumonia pleural effusion. In similar cases, the increased tumor marker values could induce investigators to continue the diagnostic iter.

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Review Article

Motilides and motilactides: design and development of motilin receptor agonists as a new class of gastrointestinal prokinetic drugs

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Introduction

Smooth muscle motor function of the gastrointestinal (GI) tract is the result of an equilibrium between stimulatory events, predominantly regulated through acetylcholine release, and inhibitory mechanisms, regulated by dopa · ine. Prokinetic drugs (1) represent a limited number of structurally unrelated compounds which induce GI smooth muscle contraction, both *in vitro* and *in vivo*. Smooth muscle motor function leading to GI motility can be stimulated both by dopamine antagonists, such as metoclopramide and domperidone, and by compounds which release acetylcholine such as cisapride. A clinically useful agent in this class of prokinetic drugs is one that not only enhances smooth muscle contraction but also coordinates such activity between different sites in the GI tract. In this respect, none of the currently available agents qualify as optimal prokinetic therapeutics, in addition to their shortcomings related to variable efficacy and endocrine/CNS side effects.

Motilides and motilactides as prokinetics are members of the macrolide family (2), which refers to a wide number of compounds containing a lactone moiety in a large ring. Erythromycin, a macrolide antibacterial, has been in clinical use for the past 40 years. However, this decade has witnessed the "renaissance" of erythromycin, related to the discovery of new pharmacological actions and not to the mechanism of its antibacterial activity. In this review, we will discuss the design, development and clinical introduction of several erythromycin derived motilides and motilactides with potential for treatment of GI motility disorders.

Discovery of motilin

During the 1960s, the laboratory of J.C. Brown (3) investigated the induction of gastric motility in the dog using duodenal alkalinization. Brown hypothesized that two possible mechanisms of action could be involved in producing the observed stimulation of motor activity: blocking the release of an inhibitory substance from the duodenal mucosa, or the release of a humoral stimulating agent. In support of the latter hypothesis, in 1971 Brown and coworkers (4, 5) isolated a humoral stimulating agent from the duodenal mucosa and 2 years later, announced the complete amino acid sequence of motilin, a 22-amino acid polypeptide (6-9) with a molecular weight of 2700 (Fig. 1).

Distribution of motilin

Motilin is found predominantly in endocrine cells of the upper small intestine (10, 11). However, the existence of motilin in the CNS and in peripheral nerves has also been

Human	Phe-Val-Pro-Ile-Phe-Thr-Tyr-Gly-Glu-Leu-Gln-Arg-Met-Glu-Glu-Lys-Glu-Arg-Asn-Lys-Gly-Gln
Pig	Phe-Val-Pro-Ile-Phe-Thr-Tyr-Gly-Glu-Leu-Gln-Arg-Met-Glu-Glu-Lys-Glu-Arg-Asn-Lys-Gly-Gln
Dog	Phe-Val-Pro-Ile-Phe-Thr-His-Ser-Glu-Leu-Lys-Arg-Ile-Arg-Glu-Lys-Glu-Arg-Asn-Lys-Gly-Gln

Note that human motilin sequence is identical to that of porcine motilin.

Fig. 1. Amino acid sequence of motilin in various species.

suggested, with extensive studies by Beinfeld having shown motilin immunoreactivity in rat cerebellum (12) at concentrations comparable to those found in intestine. Recent autoradiographic demonstration of motilin receptors in rabbit cerebellum has also been reported by Peeters' group (13). The physiological role of motilin in the brain has not, however, been clearly elucidated.

Action of motilin in the GI tract

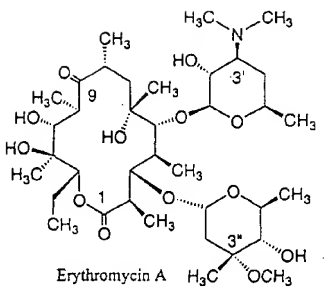
In isolated smooth muscle preparations, activity of motilin can be demonstrated in stomach and duodenum of both rabbit and humans (14-16). The action on smooth muscle appears to be direct stimulation requiring extracellular calcium (14, 15). Itoh's studies (17) have demonstrated, in both conscious dogs and human subjects, the close association of circulating motilin with the induction of phase III contraction of stomach during the interdigestive state. Soon thereafter, Itoh's laboratory described motilin stimulation of muscle contraction of the lower esophageal sphincter, sphincter of Oddi and the gallbladder. Concentrations of motilin in plasma exhibit phasic variations that are closely correlated with changes in gastric and duodenal smooth muscle motor activity (18). A rise in circulating concentration corresponds with the onset of duodenal phase III motor activity observed at regular 90- to 120-minute intervals. The phasic changes are abolished by both eating and cholinergic blockade. In addition to these findings, secretion of digestive enzymes in the stomach and pancreas has also been found to be associated with phase III smooth muscle activity, suggesting potential control by circulating motilin in both dog and man (19, 20).

Since motilin is a polypeptide, its instability at room temperature, together with a notable absence of oral efficacy, make it an unlikely candidate for commercial clinical trials. However, the recent discovery and clinical introduction of several nonpeptide motilin agonists have opened new opportunities for modulation of GI motility for the GI community.

Erythromycin as a motilin agonist

It has been known for many years that some antibiotics, including the macrolide erythromycin A (EryA), may cause GI side effects such as diarrhea, borborygmi and vomiting; however, little attention had been paid to the mechanisms involved. In 1984, two groups (21, 22), independently and nearly simultaneously, reported that EryA stimulated small intestinal contractile activity in dog. Itoh and coworkers (21) found that EryA at doses well below those required for antibacterial efficacy (0.03 mg/kg i.v.) induced a pattern of migrating contractions originating in the stomach with a frequency similar to spontaneous slow waves.

The pattern and duration of the smooth muscle activity were similar to the spontaneous activity that occurs periodically in the fasted state, *i.e.*, phase III activity of the migrating motor complex (MMC), which is a cyclical motor



Erythromycin A

pattern originating in either stomach or duodenum w migration to the terminal ileum (23). There was a striki resemblance of the EryA response to that produced motilin. Because EryA was associated with moti release in dog, Itoh suggested that the macrolide act through motilin. However, subsequent findings in m suggested EryA induced phase III activity directly with requisite motilin release (24).

Peeters *et al.* (25) subsequently demonstrated th EryA acts directly on the motilin receptor in binding exp iments using rabbit antral smooth muscle homogenat where the macrolide specifically displaced bound moti The ability of EryA to compete with motilin for comm binding sites was correlated with its potency to indu contraction in rabbit duodenal smooth muscle strips. L motilin (26), EryA had no contractile effect on mus strips of rat or dog duodenum, but did induce contracti in human strips (27).

These findings and earlier reports on antral mot receptors in man led to evaluation of the effect of int venous EryA on gastric emptying in patients with diabe gastroparesis (28), where retarded emptying of solids a liquids was markedly accelerated. Presentation of the results at the American Gastroenterological Associat meeting in Washington in 1989 marked the beginning intense research in this area and the emergence of a n class of potent prokinetic agents (motilides and motil tides) derived from erythromycin possessing improv oral activity with no antibiotic potency.

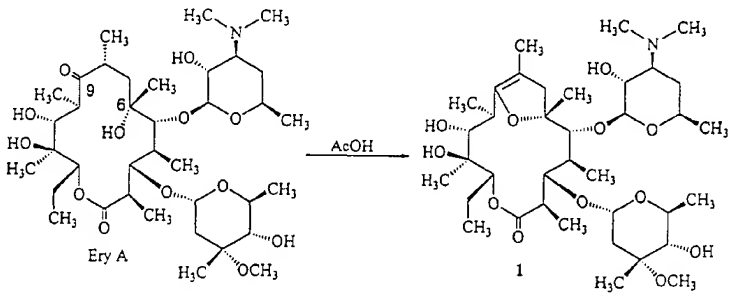
Motilides

Omura and Itoh (29) defined motilides as macroli with: a) direct contractile effect on segments of isola smooth muscle of rabbit duodenum, b) the capacity induce phase III activity in conscious dog, and c) lack antibacterial activity.

Structure-activity relationship of motilides and motilactides

Early studies in 1985 revealed that 14-membered, not 16-membered, macrolides stimulated GI contracti in conscious dogs (21, 30, 31). Two earlier studies in

Scheme 1: Synthesis of erythromycin A-6,9-hemiacetal



1960s noted that oleandomycin (14-membered macrolide) and EryA stimulated motor activity in the rabbit small intestine, gallbladder and the terminal bile duct, but both reports went largely unnoticed (32, 33). In 1992, Nakayoshi (34) included roxithromycin and clarithromycin in dog studies and found reduced smooth muscle stimulating activity relative to EryA, and further noted that all 16-membered compounds tested (josamycin, leucomycin, midecamycin and acetylspiramycin) failed to induce contractions.

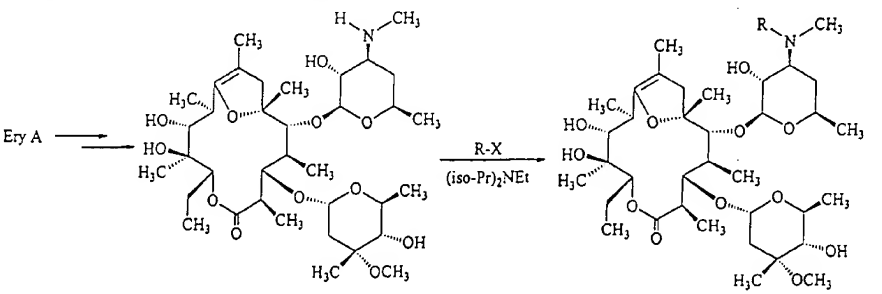
Omura *et al.* (35) demonstrated that conversion of the 9-ketone of the macrolactone ring system (Scheme 1) to an enol ether, such as **1**, led to an increase in GI motor stimulating activity (10-fold that of EryA) with a concomitant decrease in antibacterial activity. This manipulation demonstrated the feasibility of enhancing smooth muscle contractile potency with an associated decrease in antibacterial efficacy.

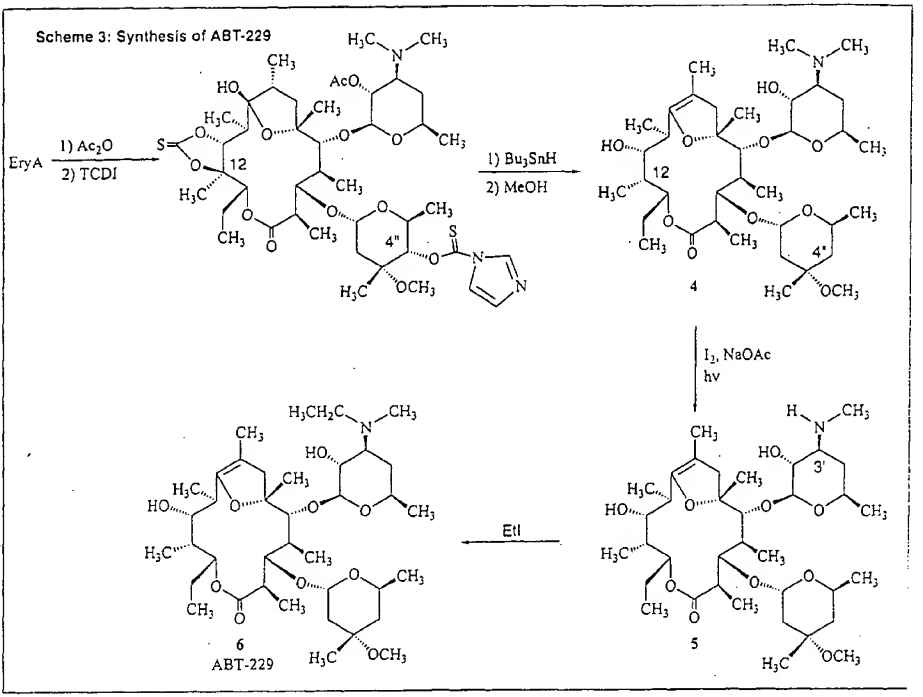
Substitutions on the 3'-position of the basic sugar (36, 37) also modulate potency (Scheme 2) in GI motility induction.

While the prokinetic potency could be increased (**2**, 18-fold and **3**, 248-fold that of EryA) with these modifications, antibacterial potency was eliminated.

Studies in our laboratories indicate that **2** and **3** have very low oral bioavailability (less than 5%) in the dog. A plausible explanation is that enol ethers of EryA, such as **2** and **3**, undergo rapid reaction involving the 12-OH to produce 6,9:9,12-spiroacetals under acidic conditions (38) in the stomach. Unlike **2** and **3**, spiroacetals have no prokinetic activity. To address this shortcoming, scientists at Abbott (39, 40) reported deoxygenation of erythromycin, at both the 12-position on the macrolactone ring and the 4"-position of the neutral sugar. These manipulations resulted in improved *in vitro* contractile potency (Scheme 3), affording an increase in prokinetic

Scheme 2: Synthesis of EM-523 and EM-574





activity which led to the identification of ABT-229 (6). In the *in vitro* rabbit duodenum contractile assay, ABT-229 was 200-fold more potent than EryA. Excellent GI stimulatory activity was also demonstrated in the conscious dog following oral administration, with contractions observed in stomach, duodenum and ileum. ABT-229 showed promising oral activity, with an ED_{50} of 0.05 mg/kg in conscious dogs. It also revealed attractive oral bioavailability in dog (25%) and an elimination half-life of 6.0 h. Like EM-523 or EM-574 (Scheme 2), ABT-229 had no antibacterial activity.

The above SAR studies also underscore the importance of the conformation of the macrolactone at C-9 to C-12 for prokinetic potency. For example, the conformation of 6, as determined by NMR, is superimposable on the X-ray crystal structure of 1. On the other hand, conformation of the 12-epi congener of 6 differs considerably in the same region (40, 41), and has been shown to be much weaker than ABT-229 in contractility studies.

In a search for greater acid stability, Koga *et al.* (42) were able to protect the 12-hydroxyl group by *O*-alkylation and found that the 12-*O*-methyl derivatives, like GM-611 (7) (Scheme 4), exhibited less lability while retaining attractive *in vitro* potency. However, compound 7 did not show improved oral bioavailability compared to ABT-229 when examined by Abbott pharmacokineticists.

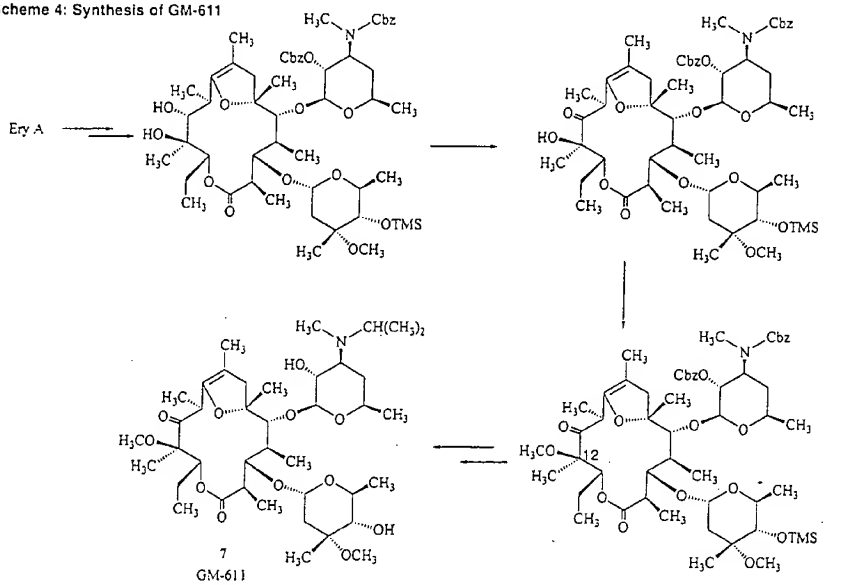
Other studies by Faghih *et al.* (43) confirmed that a key to potentiating stimulatory smooth muscle activity was to form a 6,9-epoxy ring system, as in the two epoxy derivatives 8 and 9; both compounds were more potent (220- and 240-fold, respectively) than EryA (Schemes 5 and 6).

The presence of a 6,9-cyclic ether system is more critical for smooth muscle activity than the macrolactone ring size, as shown by Abbott investigators (44, 45) in the case of a 13-membered macrolide, A-182061 (Scheme 7).

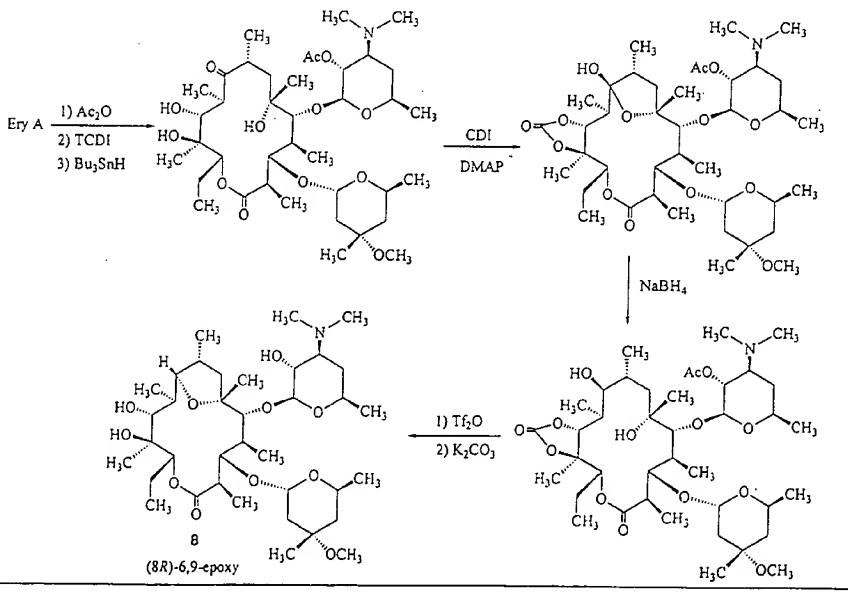
Eeckhout *et al.* (46), as well as Gregory and coworkers (47), have also confirmed that 12-membered macrolides such as KC-11458 (10) retain smooth muscle stimulating activity, although these 12- or 13-membered motilides are less potent *in vitro* than their 14-membered analogs. The 12-membered macrolides are the product o a ring rearrangement reaction of erythromycin, formed via translactonization of the 11-hydroxyl group (48).

For 14-membered 6,9-enol ether or 6,9-epoxy motilides, a conformation of the macrolactone containing a five-membered ring, which positions 11-OH close t C-1, predisposes the macrolactone ring toward intra molecular translactonization. This translactonizatio:

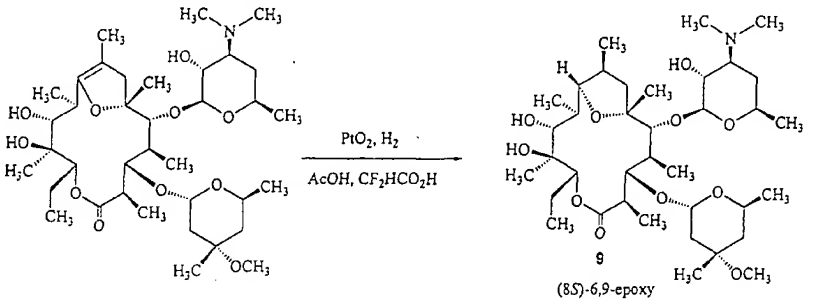
Scheme 4: Synthesis of GM-611



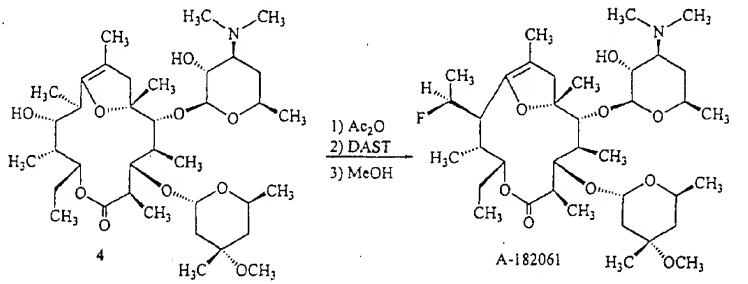
Scheme 5: Synthesis of (8*R*)-4''-deoxy-6,9-epoxyerythromycin A

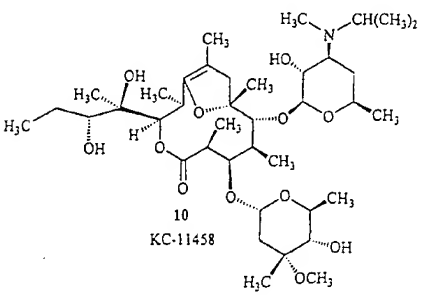


Scheme 6: Synthesis of (8*S*)-4"-deoxy-6,9-epoxyerythromycin A



Scheme 7: Synthesis of A-182061





contributes to instability of such systems. Consequently, toward the synthesis of potent and stable motilides, Abbott chemists studied the transformation of the macrolactone ring to a novel and stable macrolactam (Scheme 8).

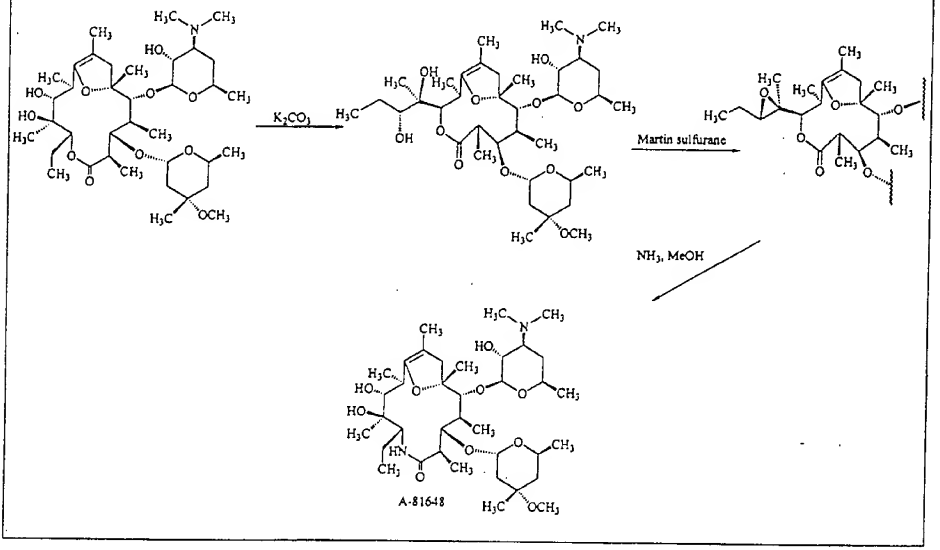
However, despite attractive *in vitro* potency (200 times that of EryA), A-81648 showed low oral bioavailability in dogs. The poor pharmacokinetic behavior may be attributed to the presence of the acid labile 6,9-enol ethe Hence, the 6,9-enol ether was replaced with an acid st ble, 6,9-epoxy with 8(*R*),9(*R*) configuration (49, 5 (Scheme 9). This transformation led to a series of cor pounds with significantly improved bioavailability. F example, A-173508 (**11**) showed excellent *in vitro* activi (330 times that of EryA), measured as isolated rabbit du denal smooth muscle contractility. Additional studi revealed oral bioavailability in dogs of 61%. A-1735(was also studied in conscious dog through measureme of GI smooth muscle contraction after oral administratio The compound demonstrated marked activity followi oral dosing with an ED_{50} of 0.036 mg/kg. Thus, A-1735(has emerged as a member of a novel and unique class potent and stable prokinetic agents, motilactides, w attractive oral bioavailability and efficacy.

Mode of action of motilides

Isolated tissue preparations

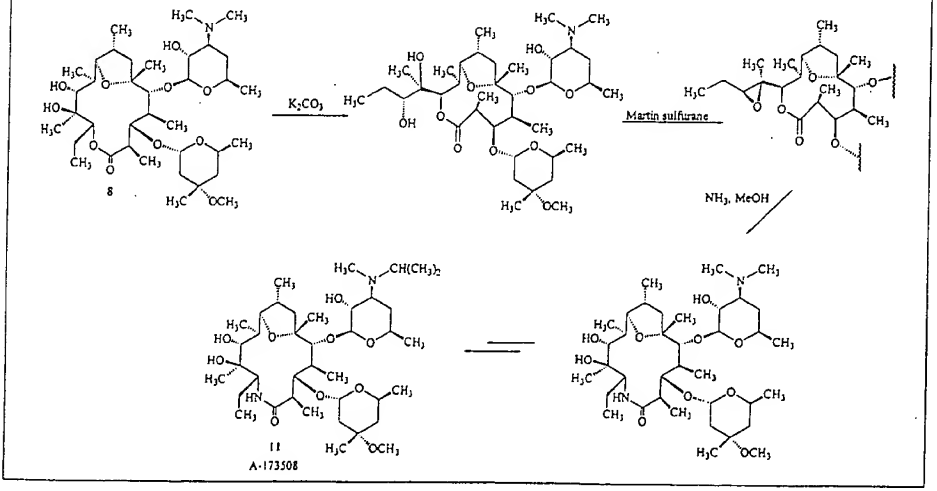
In isolated rabbit duodenal smooth muscle, motilid act directly on smooth muscle motilin receptors. Ti

Scheme 8: Synthesis of A-81648



receptor has been characterized in both contractility and binding studies (25). Early evidence that motilides are agonists for this smooth muscle receptor was recently confirmed by a report from Peeters' laboratory (51) employing a receptor antagonist. ANQ-11125, this 14-amino acid peptide derivative of motilin, at a concentration of 1 μm shifted the dose-response curves of motilin, EryA and motilides one order of magnitude, but

Scheme 9: Synthesis of A-173508



had no effect on substance P. More recently, Takanashi (52) reported the preparation of another antagonist, GM-109, a cyclic peptide derivative. Evidence has also surfaced for neurally mediated effects of motilides as reflected by the work of Morgan (53), Tack (54), Kitazawa (55) and others (56, 57). While binding studies of motilides have substantiated the presence of smooth muscle receptors, recent data from Poitras' laboratory (58) have also shown binding to synaptosomes in antral homogenates. Additional binding studies have also detected central (12, 13) neural motilin receptors. Consequently, the diversity of motilin receptor sites remains to be more rigorously characterized.

Intact animal studies

In vivo, most effects of erythromycin and motilides are neurally mediated, as shown by Ohtawa (59) for EM-523 (2) in dogs. In a series of conscious dogs implanted with force transducers, EM-523 and receptor antagonists were given intravenously during the interdigestive state. EM-523 induced phase III-like contractions in a dose-dependent manner, and the contractions were dose dependently inhibited by pretreatment with cholinergic and 5-HT_3 receptor antagonists and dopamine, but not by adrenoceptor, opiate antagonist or methysergide. Therefore, *in vivo*, EryA and motilides act on neural pathways to increase the release of acetylcholine and other excitatory transmitters. These pathways may be different in the fasting and fed states and are probably not vagally mediated, since EryA continues to accelerate gastric emptying after vagotomy in dogs (60) and man (61). Work by Morgan and Tack (53, 54) has suggested that EryA or motilin act at the level of the myenteric plexus.

These differences between *in vitro* and *in vivo* results may be due to differences in sensitivity. Recently Peeters (62) presented data suggesting that in rabbit antrum, the response toward electrical field stimulation is enhanced by motilin at much lower concentrations than those needed to induce contractions mediated via smooth muscle receptors. The neural motilin receptor may possess a higher affinity than the smooth muscle receptor, which is consistent with observations that low doses of EryA may drive neurally generated responses while high doses may directly drive muscular responses (21, 63).

Therapeutic applications of motilides

Since 1990, a large array of clinical motility disorders has been investigated using EryA as a motilin agonist with notable beneficial impact (64). With the clinical introduction of several improved erythromycin derivatives possessing improved potency and absence of antibacterial activity, the prospect of additional beneficial effects for motility disorders has been expanded.

Refux disease

Disorders of esophageal and gastric motility are the most dominant factors in the pathogenesis of gastroesophageal reflux disease (GERD). Patients with GERD have abnormal esophageal peristalsis, an incompetent lower esophageal sphincter and delayed emptying. The effect of motilides on gastric emptying and esophageal motility (66-70) suggest that motilides could find a place in the treatment of GERD. In a study with human volunteers, gastroesophageal reflux caused by white wine was reversed by EryA (71). It was also found that in patients with reflux disease, EryA shortened postprandial reflux duration. More data are needed to evaluate the potential of motilides in the treatment of gastroesophageal reflux disease.

Diabetic gastroparesis

The first demonstration of clinical utility for the prokinetic action of EryA was reported by Janssens (28). In these studies the gastric emptying of both solids and liquids was significantly accelerated in normal subjects and in patients suffering from gastroparesis due to diabetes. Intravenous, as well as orally administered (65), EryA increased emptying, and the effect was maintained for several weeks on repeated oral dosing.

Postoperative ileus

Interdigestive activity is important in postoperative patients. In postoperative ileus the frequency of phase III cycles is decreased, and it has been suggested that return of the migrating motor complex signals return to normalcy. Since motilides induce phase III activity, their potential for the treatment of this disorder is obvious. Positive results (73) have been reported with EM-523 (2).

Scleroderma

In systemic sclerosis different areas of the GI tract are affected, commonly the esophagus and small intestine and less often the stomach. When gastric emptying is delayed, migrating motor complex patterns are absent and patients develop bezoars and bacterial overgrowth. Motilides may play a role in the symptomatic treatment of this disorder (74, 75).

Gallstones

The effect on the gallbladder (76, 77) suggest that motilides may find application in patients with risk of gallstone formation, as a prophylactic to reduce recurrence or to aid fragment clearance after shock-wave lithotripsy.

Duodenal intubation

In children with the presumptive diagnosis of chronic intestinal pseudoobstruction, EryA facilitates the postpyloric passage of tubes during duodenal intubation. It is also reported that EryA facilitated the migration of antral feeding tubes (78).

Other applications

In patients with chronic idiopathic pseudoobstruction, EryA was able to induce phase III activity and improve clinical symptoms (72, 79). To empty the stomach before emergency surgery, intravenous EryA has been recommended as a quick and safe procedure (64). Also in patients with gastric stasis, intravenous EryA cleared the stomach and facilitated endoscopy (80).

Motilides in clinic

Morrison (81) studied the safety and pharmacodynamics of a single oral dose of ABT-229 (6) in 36 healthy subjects. The drug was safe and well tolerated in doses up to 64 mg and accelerated gastric emptying. Maes *et al.* (82) also studied ABT-229 in humans. Gastric emptying was evaluated using the octanoic acid breath test (83). ABT-229, after an oral dose of 4 mg, significantly increased gastric emptying of solids in man. In another study by Verhagen in 9 volunteers (84), a single oral dose of ABT-229 (4 mg) strongly stimulated postprandial antral motility. Finally, Verlinden *et al.* (85) described the dose-dependent acceleration of solid gastric emptying with ABT-229 in 132 healthy male subjects. ABT-229 accelerated gastric emptying of a solid meal in normal subjects, and was safe and well tolerated. Consistent responses across all meals required twice-daily dosing. The lowest effective dose was 2.5 mg twice daily. There was no evidence of tachyphylaxis. In light of these encouraging results, ABT-229 has entered phase IIB clinical trials.

Two other motilides from Takeda, EM-523 and EM-574, are also under clinical development. However, few clinical reports are available except for one study by Hanyu (73) and another by Nakamura *et al.* (86), showing improved gastric emptying in 6 patients with diabetic gastroparesis after intravenous administration of EM-523.

Summary

In parallel to the morphine and enkephalin relationship developed over the last 20 years, macrolide derivatives (motilides and motilactides) as highly potent agonists for receptors of the endogenous peptide, motilin, provide intriguing new possibilities for the study and treatment of motility disorders. As clinical development of this family of erythromycin derivatives continues, it is very probable that a compound with no antibacterial activity may find a major role in the therapy of gastrointestinal motility and be a useful addition to our rather limited armamentarium of effective and safe gastrointestinal prokinetic agents.

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